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FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2017/2018

Ana Teresa Pinto e Silva

Rapid flow cytometry test for identification of different
carbapenemases in Enterobacteriaceae
Teste rápido para identificação das diferentes carbapenemases
em Enterobacteriaceae por citometria de fluxo

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Mestrado Integrado em Medicina

Área: Microbiologia

Tipologia: Dissertação

**Trabalho efetuado sob a Orientação de:
Professora Doutora Cidália Irene Azevedo Pina Vaz**

**Trabalho organizado de acordo com as normas da revista:
Antimicrobial Agents and Chemotherapy**

março, 2018

FMUP

Eu, Ana Teresa Pinto e Silva, abaixo assinado, nº mecanográfico 200000044, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

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DESIGNAÇÃO DA ÁREA DO PROJECTO

Microbiologia

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Rapid flow cytometry test for identification of different carbapenemases in Enterobacteriaceae

ORIENTADOR

Professora Doutora Cidália Irene Azevedo Pina Vaz

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À minha Mãe

1 **Rapid flow cytometry test for identification of different carbapenemases in**
2 **Enterobacteriaceae**

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15 Running Title: Carbapenemases identification by flow cytometry

16

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26 **Abstract**

27 Enterobacteriaceae producing carbapenemases, such as KPC or metallo- β -
28 lactamases, have emerged on several continents. Phenotypic tests are urgently needed
29 for their rapid and accurate detection. A novel and fast flow cytometry test was
30 developed in order to identify carbapenemase production by Enterobacteriaceae and to
31 discriminate between the different types of carbapenemases belonging to Ambler
32 molecular classes A, B, and D. It is based upon the comparison of meropenem
33 antimicrobial activity alone and coupled with different carbapemase inhibitors
34 (aminophenylboronic acid and EDTA), assessed by flow cytometry. Decreased
35 susceptibility to carbapenems caused by either ESBL or AmpC enzymes combined with
36 decreased permeability was also detected using temocillin and cloxacillin, overcoming
37 the false positive results obtained with phenotypic carbapenemase detection tests. The
38 test was applied directly to 60 bacterial colonies, half of them carbapenemases
39 producers (KPC, VIM, IMP, NDM, IMI, GIM, OXA-48-like, and KPC plus VIM). The
40 test yielded sensitivity and specificity of 100%, distinguished accurately between
41 several different mechanisms mediating reduced susceptibility to carbapenems in
42 Enterobacteriaceae. It represents a convenient and reliable phenotypical approach for
43 detection and characterization of different carbapenemases with a time-to-results of 2
44 hours *versus* 24 hours of classic methods.

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51 **Introduction**

52 In the last decade, the prevalence of resistance to β -lactam antibiotics mediated
53 by β -lactamases has increased at an alarming rate, especially among Enterobacteriaceae
54 (1); firstly, because of the spread of extended spectrum β -lactamases (ESBL) and more
55 recently due to carbapenemases, which are enzymes able to hydrolyze carbapenems.
56 These enzymes have also spread rapidly throughout the world (2), being an alarming
57 problem in some countries, especially in Greece and India (3). In 2015, 13/38 countries
58 reported inter-regional spread of or an endemic situation for carbapenemase-producing
59 Enterobacteriaceae (CPE), compared with 6/38 in 2013. Only three countries replied
60 that they had not identified one single case of CPE (4). Carbapenemases include Ambler
61 class A (such as *Klebsiella pneumoniae* carbapenemase, KPC), Ambler class B
62 (metallo- β -lactamases, MBL, such as VIM-type, IMP-type, NDM-type) and Ambler
63 class D (OXA-48-types, such as OXA-181) enzymes (Table 1). Invasive infections with
64 CPE are associated with high morbidity and mortality (5, 6). Carbapenemases may
65 confer resistance to virtually all β -lactams, and are readily transferable. Additionally, CPE
66 frequently possess resistance mechanisms to a wide-range of antimicrobial agents, since
67 such enzymes hydrolyze penicillins, in most cases cephalosporins, and to various
68 degrees carbapenems and monobactams (the latter are not hydrolyzed by metallo- β -
69 lactamases). Accurate detection of CPE is relevant at individual patient not only due to
70 the impact on antibiotic therapy but also for infection control purposes, especially in
71 outbreak settings. The European Committee of Antimicrobial Susceptibility Testing
72 (EUCAST) recently developed guidelines for carbapenemase detection in
73 Enterobacteriaceae isolates (7, 8). These recommendations must be adopted whenever
74 decreased susceptibility to imipenem, meropenem and/or ertapenem is detected,
75 meaning Minimal Inhibitory Concentration (MIC) values >1 , >0.125 and >0.125 mg/L,

76 respectively; values below the clinical breakpoints for susceptible phenotype. In the
77 most recently recommendations (8), imipenem was not considered and meropenem is
78 the carbapenem that offers the best compromise between sensitivity and specificity in
79 terms of detecting carbapenemase-producers (9). However, it usually requires 48 hours
80 after sample collection to determine the MIC value and an additional 18-24 hours to
81 confirm the presence of carbapenemase. Combination disk test is the unique most
82 convenient phenotypic method to discriminate different carbapenemases (10-12). In
83 brief, disks or tablets containing meropenem without and with various inhibitors such as
84 aminophenyl boronic acid (APBA) as a class A inhibitor and ethylenediaminetetraacetic
85 acid (EDTA) as a class B inhibitor are used. No inhibitor to OXA-48-type enzymes was
86 included in the phenotypic panels. However, these enzymes confer high-level
87 resistance to temocillin (12). Decreased susceptibility of Enterobacteriaceae to
88 carbapenems may be also caused by either the production extended spectrum β -
89 lactamases (ESBL) or AmpC β -lactamases coupled with decreased permeability due to
90 alteration or down-regulation of porins (13). Cloxacillin, which inhibits AmpC β -
91 lactamases, should be added to the test in order to differentiate between AmpC
92 hyperproduction plus porin loss and carbapenemase-production. Figure 1 represents an
93 algorithm for interpretation of results with β -lactamases inhibitors recommended by
94 EUCAST. The main disadvantage of such procedure is that it requires a long incubation
95 period (18-24 hours), since it is based on growth in presence of the drugs. The same
96 drawback is appointed to Etest KPC and MBL strips, which associate a carbapenem
97 with boronic acid or EDTA, respectively (14). A possible alternative is the carbapenem
98 inactivation method (i.e. CIM-test), which has had variable performance in different
99 studies and requires at least 18 hours to the result (15). The market available
100 chromogenic culture media although easy to perform are also growth dependent (16).

101 More recently, different biochemical test such as Carba NP was described to detect
102 carbapenemase production (17, 18); it seems to be rapid (<2h), sensitive and specific
103 although cumbersome and expensive and a discrimination among carbapenemase-types
104 is not possible. MALDI-TOF MS, recently introduced in the laboratory routine, seems
105 promising in order to detect antibiotic modifications due to degradation by enzymes,
106 including carbapenemases (19, 20). A new immunochromatographic lateral flow assay
107 is available for direct identification OXA-48-like enzymes and KPC, but its robustness
108 has not been evaluated so far (21). Molecular identification of carbapenemase genes
109 (22, 23) is another possibility but, besides the several genes that must be searched and
110 high associated costs, it can result in false negatives when mutations or new genes
111 emerge.

112 Flow cytometry (FC) represents an accurate and fast approach for the analysis of
113 cell architecture and its functional parameters, with considerable advantages over
114 conventional methods (24). Similar to ESBL detection by FC (25), a novel protocol for
115 carbapenemase detection, with time-to-results within 2 hours, was hereby developed
116 and evaluated, using a comprehensive set of phenotypically and molecularly well-
117 characterized strains.

118

119 **Results**

120 An increase (of at least twice) in fluorescence intensity, meaning cell lesion, in
121 the presence of inhibitors, when compared with the intensity in the presence of
122 meropenem alone, was observed with APBA for all KPC, corresponding to class A
123 carbapenemases. All VIM, IMP, NDM, IMI and GIM showed an increase in intensity of
124 fluorescence when cells were treated with meropenem associated with EDTA,
125 corresponding to class B carbapenemases. Strains with both KPC and MBL enzymes

126 had an increase in intensity of fluorescence when cells were treated with meropenem
127 associated only with APBA plus EDTA. For class D carbapenemases no increase was
128 observed however, they were resistant to temocillin; the fluorescence intensity of cells
129 exposed to temocillin was similar to non-exposed cells. For AmpC associated with
130 porin loss, the increase in cells intensity of fluorescence was observed with APBA but
131 also with cloxacillin. In case of ESBL associated with porin loss no increase in intensity
132 of fluorescence was observed; such strains were susceptible to temocillin, increasing
133 fluorescence intensity of cells exposed to this drug. In Figure 2 a graphic showing the
134 median values of intensity of fluorescence for each type of carbapenemases and for a
135 group of non-producers is represented. A typical example of the different types
136 concerning flow cytometry data are shown in figure 2. This FC kit combines excellent
137 sensitivity (100%), specificity (100%) and short time to result.

138

139 **Discussion**

140 Carbapenemases are considered to be of high epidemiological importance,
141 particularly when they confer decreased susceptibility to carbapenems. It is high
142 recommended the application in clinical routine of phenotypic methods for detection of
143 carbapenemases. The main categories of methods available have disadvantages
144 encouraging the development of novel rapid methods. The rapid phenotypic tests been
145 so far developed have limitations. The colorimetric tests (CarbaNP test, Blue-Carba test
146 or β CARBA testTM) only detect the carbapenemase production not allowing the
147 distinction among the different classes and lack sensitivity for Enterobacteriaceae
148 producing OXA-48 (17, 26, 27). Similar disadvantages are also appointed to MALDI-
149 TOF technology for detection of carbapenem hydrolysis (19, 20). A new
150 immunochromatographic lateral flow assay although promising its robustness has not

151 been evaluated so far (21, 28). FC already demonstrated to be an excellent tool in
152 microbiology (24), overcoming the methods in use in routine clinical laboratories
153 depending on growth; it gives a quicker and much more informative antimicrobial
154 phenotype. The present work described the performance of a new method based upon
155 FC that enables the fast determination of carbapenemases. The test exhibited 100% of
156 sensitivity and specificity directly from bacterial colonies. It has the potential to be
157 applied directly from blood cultures, saving extra 24 hours for bacterial isolation after
158 sample collection. Indeed, a novel FASTinov[®] kit for antimicrobial susceptibility
159 testing (AST) of Gram negative bacilli directly on positive blood culture was already
160 tested and exhibited an overall agreement of 98% with the reference microdilution
161 method (29). Moreover, the impact of the use of a rapid AST in clinical routine was
162 evaluated resulting in a reduced in hospital length of stay, lower consumption of
163 antibiotics and in ultimately case improved infection control. Recently, new data was
164 obtained with the use of this methodology directly from blood culture. The kit was able
165 to distinct the different types of carbapenemases (30).

166 The method although currently present as a semi-automated method, since it has
167 dedicated software, it could be fully automatized. The estimated costs for this new
168 method are rather low, around two and half euros per microorganism and the FC
169 equipment is frequently found in most hematology/immunology laboratories. The early
170 detection of carbapenemases could have a real impact on outbreaks as well as in
171 antibiotic therapy (31, 32). The improvement in detection and surveillance of CPE
172 might contribute for their epidemiological control.

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176 **Material and Methods**

177 Bacterial strains

178 A panel of 30 carbapenemase-producing Enterobacteriaceae strains was used in this
179 study: KPC (8), VIM (7), IMP (1), NDM (4), IMI (1), GIM (1), OXA-48-like (6), and
180 KPC plus VIM (2). As non-carbapenemase producers, 30 Enterobacteriaceae strains,
181 including several strains that give false positive results on phenotypic tests (9), like
182 ESBL or AmpC producers coupled to impermeability, were included.

183 Inoculum preparation

184 Bacterial suspensions, of the 60 strains described above, were prepared in Brain-Heart
185 medium (Merck) with approximately 10^6 cells/mL at initial log phase, ($O.D._{600nm} \geq 0.2$,
186 which takes approximately 1.5 hours).

187 Flow cytometric kit inoculation

188 A novel flow cytometric kit for carbapenemase detection (provided by FASTinov Ltd,
189 Porto, Portugal) was used. Briefly, bacterial cells obtained directly from colonies were
190 incubated with meropenem (2 and 8 $\mu\text{g/mL}$) with and without the inhibitors APBA (2.5
191 and 5 mM), EDTA (6.25 and 12.5 mM), APBA plus EDTA (2.5 plus 6.25 and 5 plus
192 12.5 mM) and cloxacillin (250 and 500 $\mu\text{g/mL}$). Temocillin (Eumedica S.A., Brussels,
193 Belgium) alone, at 32 and 64 $\mu\text{g/mL}$ was also included. The kit fluorochrome dye was
194 disposed in all tubes. Cells were incubated for 1 hour at 37°C and analyzed in a flow
195 cytometer apparatus.

196 Flow cytometry analysis

197 Bacteria cells after exposure to antimicrobial treatments were analysed at a
198 FACSCalibur flow cytometer (Becton Dickinson). The intensity of fluorescence of cells
199 treated with meropenem alone was compared with those exposed to meropenem plus
200 inhibitors.

201 **Acknowledgments**

202 We would like to thanks FASTinov S.A. (Porto, Portugal) for providing the FC kits and
203 to Helena Ramos, MD, (Centro Hospitalar do Norte, Porto, Portugal) for providing
204 strains. We are grateful to Isabel Santos for excellent technical assistance.

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350 **TABLE 1.** Classification of carbapenemases according to Ambler molecular classes.

351

Molecular class	Enzyme
A	KPC, NMC, IMI, SME, GES
B	IMP, VIM, GIM, SPM, NDM
D	OXA

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370 **Figure 1.** Algorithm for interpretation of phenotypic tests for carbapenemase detection
371 with β -lactamases inhibitors. APBA, aminophenylboronic acid; PBA, phenyl boronic
372 acid; EDTA, ethylenediaminetetraacetic acid; DPA, dipicolinic acid; MBL, metallo- β -
373 lactamase; KPC, *Klebsiella pneumoniae* carbapenemase. *MIC above the
374 epidemiological cut-off values defined by EUCAST.

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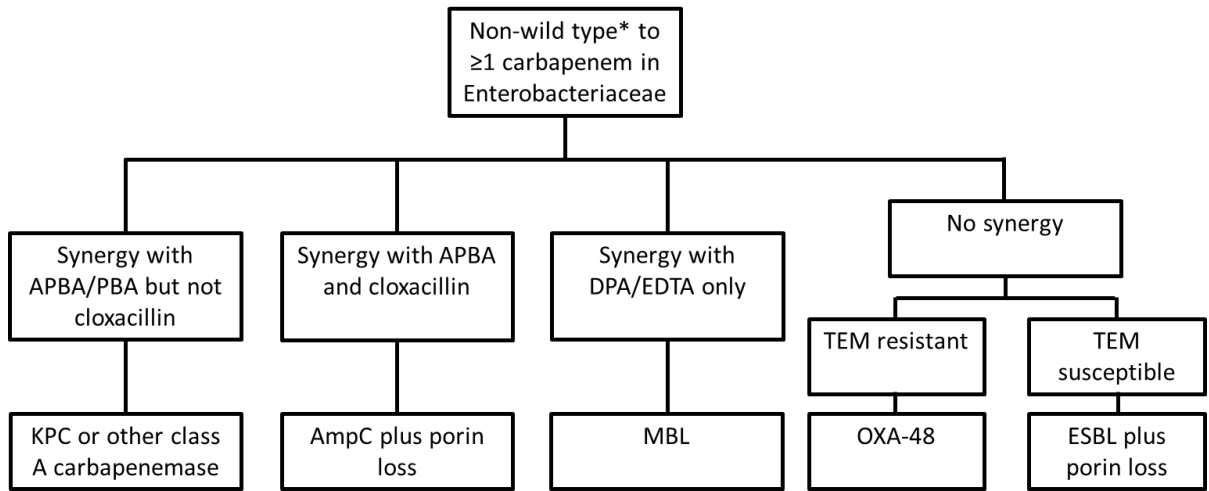
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413 **Figure 2.** Graphic representing the mean of stain indexes of each group of strains: KPC
414 carbapenemases, MBL carbapenemases, OXA-48-like carbapenemases and non-
415 producers, being the stain index the ratio between the intensity of fluorescence at FL1 of
416 cells treated with the meropenem plus the inhibitor(s) and cells treated only with
417 meropenem. Concerning temocillin the stain index is the ratio between cells treated with
418 temocillin and cells no treated. For each strain the combination both drugs at lower
419 concentration each was evident an increase in intensity of fluorescence was choose to
420 performed the calculation.

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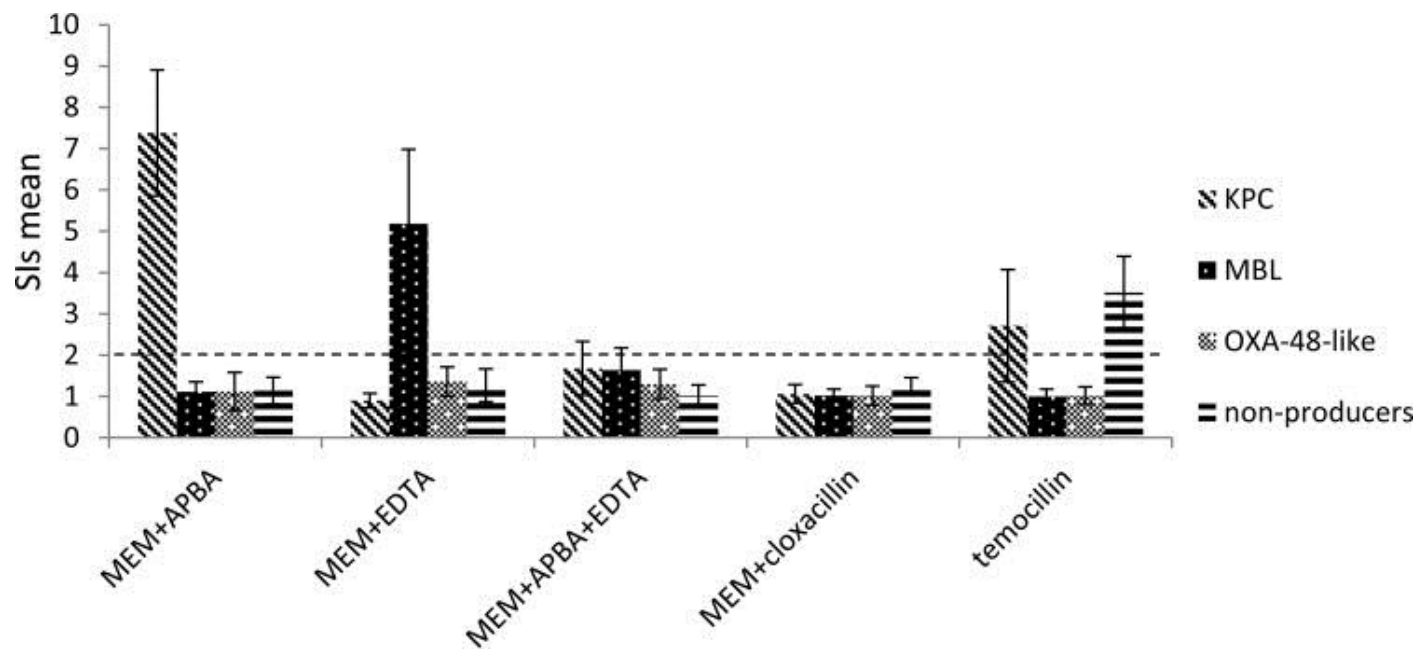
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441 **Figure 3.** Representative histograms obtained by flow cytometry for different type of
442 carbapenemases. For each strain (*Klebsiella pneumoniae* KPC-2, *Enterobacter cloacae*
443 VIM-4 and *K. pneumoniae* OXA-48) it is represented the cells non-exposed to drugs,
444 cells exposed to meropenem 8 µg/mL and with the inhibitors APBA 5 mM, EDTA 12,5
445 mM, APBA 5 mM plus EDTA 12,5 mM or cloxacillin 500 µg/mL and cells exposed to
446 temocillin 32 µg/mL. IF, intensity of fluorescence.

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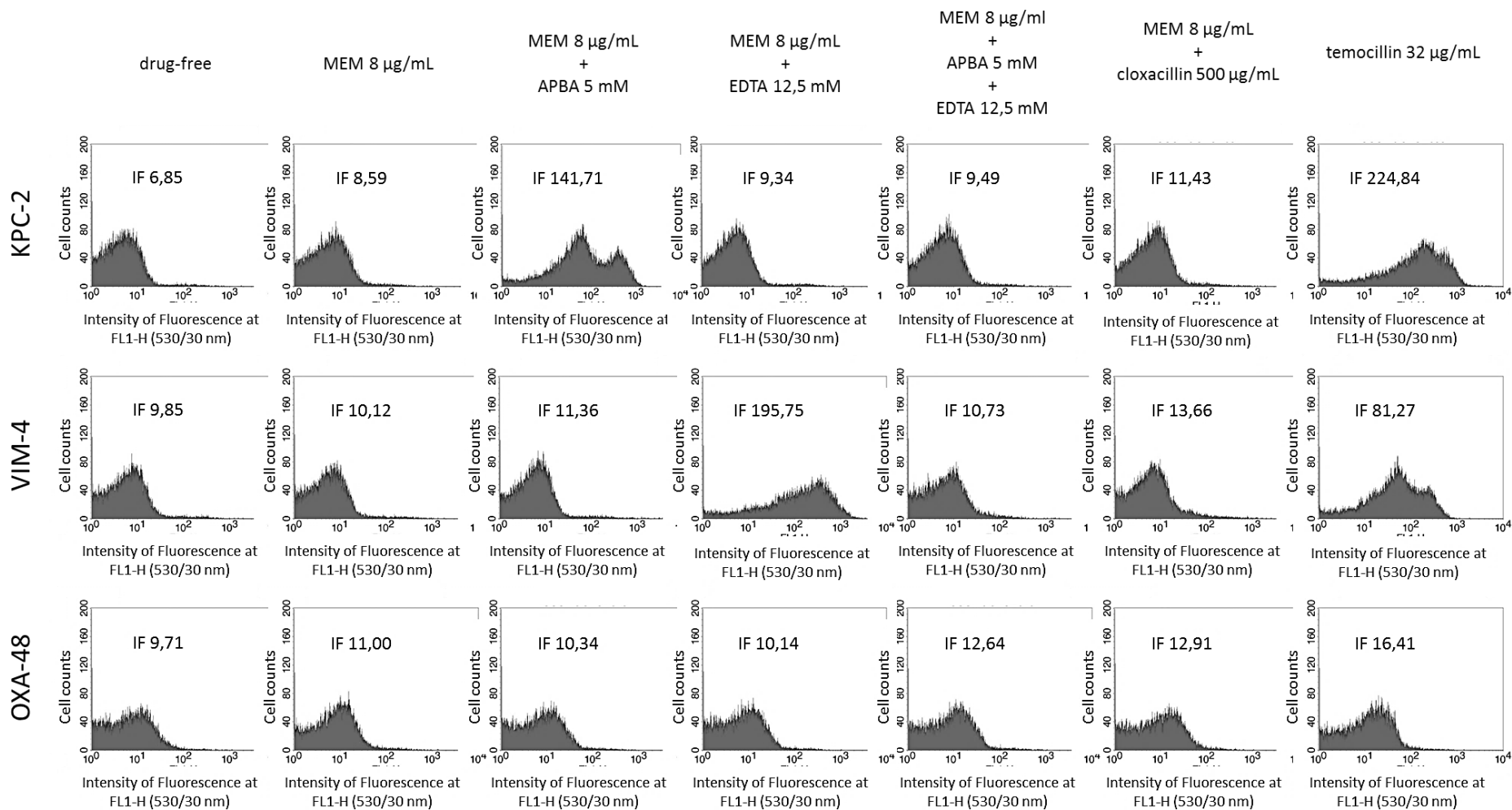
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Agradecimentos

Os meus sinceros agradecimentos,

À Professora Doutora Cidália Pina Vaz, orientadora do trabalho, pela partilha da sua sabedoria, pelas constantes palavras de incentivo e carinho e pelo seu apoio em momentos marcantes do meu percurso académico e pessoal.

Ao Professor Doutor Acácio Rodrigues, pelo seu contributo neste trabalho, pela amizade e pela confiança que em mim deposita.

Ao Grupo de Investigação da Microbiologia, pela colaboração neste trabalho, pelo apoio, companheirismo e pela partilha de bons momentos.

À Minha Família, em especial aos meus meninos, Miguel e Gabriel, pelo amor que nos une.

À minha doce Mãe e a Mãe de todos nós, Maria, que me amparam e ajudam a trilhar o meu caminho.

Normas da Revista

Antimicrobial Agents and Chemotherapy

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SCOPE

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8. Fitzgerald G, Shaw D. *In* Waters AE (ed), *Clinical microbiology*, in press. EFH Publishing Co, Boston, MA.* {Chapter title is optional.}
9. Green PN, Hood D, Dow CS. 1984. Taxonomic status of some methylotrophic bacteria, p 251–254. *In* Crawford RL, Hanson RS (ed), *Microbial growth on C₁ compounds*. Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, DC.
10. Rotimi VO, Salako NO, Mohaddas EM, Philip LP. 2005. Abstr 45th Intersci Conf Antimicrob Agents Chemother, abstr D-1658. {Abstract title is optional.}
11. Smith D, Johnson C, Maier M, Maurer JJ. 2005. Distribution of fimbrial, phage and plasmid associated virulence genes among poultry *Salmonella enterica* serovars, abstr P-038, p 445. Abstr 105th Gen Meet Am Soc Microbiol. American Society for Microbiology, Washington, DC. {Abstract title is optional.}

12. García CO, Paira S, Burgos R, Molina J, Molina JF, Calvo C, Vega L, Jara LJ, García-Kutzbach A, Cuellar ML, Espinoza LR. 1996. Detection of *Salmonella* DNA in synovial membrane and synovial fluid from Latin American patients using the polymerase chain reaction. *Arthritis Rheum* 39(Suppl 9):S185. {Meeting abstract published in journal supplement.}
13. O'Malley DR. 1998. PhD thesis. University of California, Los Angeles, CA. {Title is optional.}
14. Stratagene. 2006. Yeast DNA isolation system: instruction manual. Stratagene, La Jolla, CA. {Use the company name as the author if none is provided for a company publication.}
15. Odell JC. April 1970. Process for batch culturing. US patent 484,363,770. {Include the name of the patented item/process if possible; the patent number is mandatory.}
16. Harrison F, Roberts AEL, Gabriliska R, Rumbaugh KP, Lee C, Diggle SP. 2015. A 1,000-year-old antimicrobial remedy with antistaphylococcal activity. *mBio* 6:e01129-15. {Original article that describes how data submitted to a database were generated.}
17. Harrison F, Roberts AEL, Gabriliska R, Rumbaugh KP, Lee C, Diggle SP. 2015. Data from "A 1,000-year-old antimicrobial remedy with antistaphylococcal activity." Dryad Digital Repository <https://doi.org/10.5061/dryad.mn17p>. {Citation for the database where the data in the previous reference were deposited; the URL is necessary.}
18. Wang Y, Rozen D. 2016. Colonization and transmission of the gut microbiota of the burying beetle, *Nicrophorus vespilloides*, through development. *bioRxiv* <https://doi.org/10.1101/091702>.

*A reference to an in-press ASM publication should state the control number (e.g., AAC00123-18) if it is a journal article or the name of the publication if it is a book.

In some online journal articles, posting or revision dates may serve as the year of publication; a DOI (preferred) or URL is required for articles with nontraditional page numbers or electronic article identifiers.

Magalon A, Mendel RR. 15 June 2015, posting date. Bio-synthesis and insertion of the molybdenum cofactor. *EcoSal Plus* 2015 doi:10.1128/ecosalplus.ESP-0006-2013.

Note: a posting or accession date is required for any online reference that is periodically updated or changed.

Citations of ASM Accepts manuscripts should look like the following example.

Wang GG, Pasillas MP, Kamps MP. 15 May 2006. Persistent transactivation by Meis1 replaces Hox function in myeloid leukemogenesis models: evidence for co-occupancy of Meis1-Pbx and Hox-Pbx complexes on promoters of leukemia-associated genes. *Mol Cell Biol* doi:10.1128/MCB.00586-06.

Other journals may use different styles for their publish-ahead-of-print manuscripts, but citation entries must include the following information: author name(s), posting date, title,

journal title, and volume and page numbers and/or DOI. The following is an example:

Zhou FX, Merianos HJ, Brunger AT, Engelman DM. 13 February 2001. Polar residues drive association of poly-leucine transmembrane helices. *Proc Natl Acad Sci U S A* doi:10.1073/pnas.041593698

To encourage data sharing and reuse, ASM recommends reporting data sets and/or code both in a dedicated "Data availability" paragraph and in References. The components of a complete data citation include the following:

- Responsible party (senior author, collector, agency),
- Publication year,
- Complete name of a data set, including the name of the database or repository and its URL, **or** the name of the analysis software (if appropriate), including the version and project,
- Publisher (if appropriate), and
- Persistent unique identifier(s) (e.g., URL[s] or accession number[s]).

The following templates may be helpful.

Author. Year. Description of study topic. Retrieved from Database URL (accession no. ●●●●●●). {Unpublished raw data.}

Author. Year. Description or title of software (version). Repository URL. Retrieved day month year. {Software or code.}

Examples follow.

Christian SL, McDonough J, Liu C-Y, Shaikh S, Vlamakis V, Badner JA, Chakravarti A, Gershon ES. 2002. Data from "An evaluation of the assembly of an approximately 15-Mb region on human chromosome 13q32-q33 linked to bipolar disorder and schizophrenia." GenBank <https://www.ncbi.nlm.nih.gov/nucleotide/AF339794> (accession no. AF339794). {Accession number.}

Sun Z. 2013. Reprocessed: in-depth membrane proteomic study of breast cancer tissues. ProteomeXchange <http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=RPXD000665> (accession number requested). {Unsigned accession number.}

Hogle S. 2015. Supplemental material for Hogle et al. 2015 *mBio*. figshare <https://doi.org/10.6084/m9.figshare.1533034.v1>. Retrieved 16 March 2017. {Code and/or software.}

Nesbitt HK, Moore JW. 2016. Data from "Species and population diversity in Pacific salmon fisheries underpin indigenous food security." Dryad Digital Repository <https://doi.org/10.5061/dryad.ng8pf>. {Data set in repository.}

Manuscript submissions that have appeared in preprint archives should cite the preprint in References, and the fact that a paper has appeared online before should be mentioned parenthetically at the end of the introductory section: (This article was submitted to an online preprint archive [1].) The reference should take the form noted above in reference 18.

(ii) **References cited in the text.** References that should be cited in the text include the following:

- Unpublished data
- Manuscripts submitted for publication
- Unpublished conference presentations (e.g., a report or poster that has not appeared in published conference proceedings)
- Personal communications
- Patent applications and patents pending
- Websites

These references should be made parenthetically in the text as follows:

- ... similar results (R. B. Layton and C. C. Weathers, unpublished data).
- ... system was used (J. L. McNerney, A. F. Holden, and P. N. Brighton, submitted for publication).
- ... as described previously (M. G. Gordon and F. L. Rattner, presented at the Fourth Symposium on Food Microbiology, Overton, IL, 13 to 15 June 1989). *{For non-published abstracts and posters, etc.}*
- ... this new process (V. R. Smoll, 20 June 1999, Australian Patent Office). *{For non-U.S. patent applications, give the date of publication of the application.}*
- ... as suggested by the World Health Organization (<http://www.who.int/campaigns/immunization-week/2017/en/>).

URLs for companies that produce any of the products mentioned in your study or for products being sold may not be included in the article. However, company URLs that permit access to scientific data related to the study or to shareware used in the study are permitted.

(iii) **Citations in abstracts.** Because the abstract must be able to stand apart from the article, references cited in it should be clear without recourse to the References section. Use an abbreviated form of citation, omitting the article title, as follows.

- (P. S. Satheshkumar, A. S. Weisberg, and B. Moss, *J Virol* 87:10700–10709, 2013, doi:10.1128/JVI.01258-13)
- (J. H. Coggin, Jr., p. 93–114, in D. O. Fleming and D. L. Hunt, ed., *Biological Safety. Principles and Practices*, 4th ed., 2006)
- “... in a recent report by D. A. Hopwood (mBio 4:e00612-13, 2013, doi:10.1128/mBio00612-13)”

This style should also be used for Addenda in Proof.

(iv) **References related to supplemental material.** If refer-

ences must be cited in the supplemental material, list them in a **separate** References section within the supplemental material and cite them by those numbers; do not simply include citations of numbers from the reference list of the associated article. If the same reference(s) is to be cited in both the article itself and the supplemental material, then that reference would be listed in both References sections.

Short-Form Papers

The Short-Form format is intended for the presentation of brief observations that do not warrant full-length Research Articles. Submit Short-Form papers in the same way as Research Articles. They receive the same review, they are not published more rapidly than Research Articles, and they are not considered preliminary communications.

The title, running title (not to exceed 54 characters and spaces), byline, and correspondent footnote should be prepared as for a Research Article. Each Short-Form paper must have an abstract of no more than 75 words. Do not use section headings in the body of the paper; combine methods, results, and discussion in a single section. Paragraph lead-ins are permissible. The text should be kept to a minimum and if possible should not exceed 1,000 words; the number of figures and tables should also be kept to a minimum. Materials and methods should be described in the text, not in figure legends or table footnotes. Present acknowledgments as in Research Articles. The References section is identical to that of Research Articles.

Minireviews

Minireviews are brief (**limit of 6,000 words exclusive of references**) biographical profiles, historical perspectives, or summaries of developments in fast-moving areas of chemotherapy. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of AAC. For example, subject matter may range from structure-activity correlates among a group of semisynthetic cephalosporins to the comparative efficacies of new and old drugs in the prevention or treatment of diseases of microbial origin in humans.

Minireviews may be either solicited or proffered by authors responding to a recognized need. Irrespective of origin, Minireviews are subject to review and should be submitted via the eJP online manuscript submission and peer review system. The cover letter should state whether the article was solicited and by whom.

Minireviews must have abstracts. Limit the abstract to 250 words or fewer. The body of the Minireview may have section headings and/or paragraph lead-ins.

Author bios. At the editor's invitation, corresponding authors of minireviews may submit a short biographical sketch and photo for each author for publication with the article. Biographical information should be submitted at the modification stage.

- The text limit is 150 words for each author and should include WHO you are (your name), WHERE you received your education, WHAT positions you have held and at WHICH institutions, WHERE you are now

(your current institution), WHY you have this interest, and HOW LONG you have been in this field.

- The photo should be a black-and-white head shot of passport size. Photos will be reduced to approximately 1.125 inches wide by 1.375 inches high. Photos must meet the production criteria for regular figures and should be checked for production quality by using Rapid Inspector, provided at the following URL: <http://rapidinspector.cadmus.com/RapidInspector/zmw/index.jsp>.
- To submit, upload the text and photos with your modified manuscript in the eJP online manuscript submission and peer review system. Include the biographical text after the References section of your manuscript, in the same file. Upload the head shots in the submission system as a “Minireview Bio Photo”; **include the author’s name or enough of it for identification in each photo’s file name.**

Contact the [scientific editor](#) if you have questions about what to write. Contact the [production editor](#) if you have questions about submitting your files.

Commentaries

Commentaries are invited communications concerning topics relevant to the readership of AAC and are intended to engender discussion. Reviews of the literature, methods and other how-to papers, and responses targeted at a specific published paper are not appropriate. Commentaries are subject to review.

The length may not exceed 4,000 words, and the format is like that of a Minireview (see above) except that the abstract is limited to 75 words.

Challenging Clinical Cases in Antimicrobial Resistance

Challenging Clinical Cases are brief articles designed to familiarize and provide guidance to the reader on the clinical approach to the treatment of real, challenging cases involving multidrug-resistant organisms (bacteria, viruses, fungi, and parasites). This section is focused on providing an up-to-date scientific rationale for choosing specific antimicrobials based on available clinical, microbiological, and pharmacological data and on discussing the impact of mechanisms of resistance on the outcomes for infected patients. These articles may discuss novel therapeutic strategies for treating patients infected with multidrug-resistant organisms. Only highly interesting cases that have important mechanistic and epidemiological or novel microbiological insights will be selected for review.

The article should include (i) a brief abstract (limit of 75 words); (ii) a case section describing a single clinical case up to the point when the organism is isolated, characterization of the organism, and information about susceptibility testing, when appropriate; (iii) interesting photos, figures, and/or tables (limit of two combined) highlighting the clinical presentation (see “[Illustrations and Tables](#)” below for guidelines on acceptable file types, resolution, size, etc.); (iv) a single multiple-choice question addressing the most relevant therapeutic issues (How would you interpret the susceptibility report?

Which antimicrobials would be best for the patient presented in the case and why? What are the underlying mechanisms of resistance? Are there any particular pharmacological strategies, in terms of drug administration, delivery, etc., that could help in treating this patient?) with several possible answers as choices; (v) a description of the treatment strategy and patient outcome; and (vi) a reference list containing no more than 10 references. Sections ii and v above (case presentation and strategy/outcome) must not exceed 1,200 words combined.

An expert in the field (a reviewer) will discuss the case in a brief commentary section and explore answers to the questions posed by the author. (The commentator’s name and role will appear at the end of the published article byline.)

These articles will be made freely available to readers at the time of publication. No page charges will be associated with these articles, but the standard fee for accepted supplemental material, if any, applies. In an attempt to stimulate conversations and engagement, readers will be able to add comments via an online feature.

Letters to the Editor

Two types of Letters to the Editor may be submitted. The first type (Comment Letter) is intended for comments on final, typeset articles published in the journal (not on accepted manuscripts posted online) and must cite published references to support the writer’s argument. The second type (New-Data Letter) may report new, concise findings that are not appropriate for publication as Research Articles or Short-Form papers.

Letters may be **no more than 500 words long and must be typed double-spaced**. Refer to a recently published Letter for correct formatting. Note that authors and affiliations are listed below the title.

All Letters to the Editor must be submitted electronically, and the type of Letter (New Data or Comment) must be selected from the choices in the submission form. For Letters commenting on published articles, the cover letter should state the volume and issue in which the article was published, the title of the article, and the last name of the first author. In the Abstract section of the submission form, put “Not Applicable.” Letters to the Editor do not have abstracts. Both types of Letter must have a title, which must appear on the manuscript and on the submission form. Figures and tables should be kept to a minimum.

If the Letter is related to a published article, it will be sent to the editor who handled the article in question. If the editor believes that publication is warranted, he/she will solicit a reply from the corresponding author of the article and give approval for publication.

New-Data Letters will be assigned to an editor according to subject matter and will be reviewed by that editor and/or a reviewer.

Please note that some indexing/abstracting services do not include Letters to the Editor in their databases.

Errata

Errata provide a means of correcting errors that occurred during the writing, typing, editing, or publication (e.g., a

misspelling, a dropped word or line, or mislabeling in a figure) of a published article. Submit Errata via the eJP online manuscript submission and peer review system (see “[Submission, Review, and Publication Processes](#)”). In the Abstract section of the submission form (a required field), put “Not Applicable.” Upload the text of your Erratum as a Microsoft Word file. Please see a recent issue for correct formatting.

Author Corrections

Author Corrections provide a means of correcting errors of omission (e.g., author names or citations) and errors of a scientific nature that do not alter the overall basic results or conclusions of a published article (e.g., an incorrect unit of measurement or order of magnitude used throughout, contamination of one of numerous cultures, or misidentification of a mutant strain, causing erroneous data for only a [noncritical] portion of the study). Note that the addition of new data is not permitted.

For corrections of a scientific nature or issues involving authorship, including contributions and use or ownership of data and/or materials, all disputing parties must agree, in writing, to publication of the Correction. For omission of an author's name, letters must be signed by the authors of the article and the author whose name was omitted. The editor who handled the article will be consulted if necessary.

Submit an Author Correction via the eJP online manuscript submission and peer review system (see “[Submission, Review, and Publication Processes](#)”). Select Author Correction as the manuscript type. In the Abstract section of the submission form (a required field), put “Not Applicable.” Upload the text of your Author Correction as a Microsoft Word file. Please see a recent issue for correct formatting. Signed letters of agreement must be supplied as supplemental material not for publication (scanned PDF files).

Retractions

Retractions are reserved for major errors or breaches of ethics that, for example, may call into question the source of the data or the validity of the results and conclusions of an article. Submit Retractions via the eJP online manuscript submission and peer review system (see “[Submission, Review, and Publication Processes](#)”). In the Abstract section of the submission form (a required field), put “Not Applicable.” Upload the text of your Retraction as a Microsoft Word file. Letters of agreement signed by all of the authors must be supplied as supplemental material not for publication (scanned PDF files). The Retraction will be assigned to the editor in chief of the journal, and the editor who handled the paper and the chairperson of the ASM Journals Board will be consulted. If all parties agree to the publication and content of the Retraction, it will be sent to the Journals Department for publication.

CrossMark

ASM has implemented CrossMark. CrossMark is a multi-publisher initiative to provide a standard way for readers to locate the current version of an article. Clicking on the Cross-

Mark logo will indicate whether an article is current or whether updates have been published. Additional information about CrossMark can be found on CrossMark's [website](#) and on ASM's CrossMark [policy page](#).

ILLUSTRATIONS AND TABLES

Illustrations

Image manipulation. Digital images submitted for publication may be inspected by ASM production specialists for any manipulations or electronic enhancements that may be considered to be the result of scientific misconduct based on the guidelines provided below. Any images/data found to contain manipulations of concern will be referred to the editor in chief, and authors may then be requested to provide their primary data for comparison with the submitted image file. Investigation of the concerns may delay publication and may result in revocation of acceptance and/or additional action by ASM.

Linear adjustments to contrast, brightness, and/or color are generally acceptable, as long as the measures taken are necessary to view elements that are already present in the data and the adjustments are applied to the entire image and not just specific areas. Unacceptable adjustments to images include, but are not limited to, the removal or deletion, concealment, duplication (copying and pasting), addition, selective enhancement, or repositioning of elements within the image.

Nonlinear adjustments made to images, such as changes to gamma settings, should be fully disclosed in the figure legends at the time of submission. In addition, images created by compiling multiple files, including noncontiguous portions of the same image, should clearly convey that these multiple files are not a single image. This can be done by “[tooling](#),” or [inserting thin lines](#), between the individual images.

File types and formats. Illustrations may be continuous-tone images, line drawings, or composites. Color graphics may be submitted. Suggestions about how to ensure accurate color reproduction are given below.

On initial submission, figures may be uploaded as individual PDF files or combined and uploaded as a single PDF file. Place each legend in the text file, as well as on the same page with the corresponding figure to assist review. At the modification stage, production-quality digital files must be provided. Because the legends will be copyedited and typeset for final publication, they should appear within the main text, after the References section, and should not be included as part of the figure itself at this stage. All graphics submitted with modified manuscripts must be bitmap, grayscale, or in the RGB (preferred) or CMYK color mode. See “[Color illustrations](#).” Half-tone images (those with various densities or shades) must be grayscale, not bitmap. AAC accepts TIFF or EPS files but discourages PowerPoint for either black-and-white or color images.

For instructions on creating acceptable EPS and TIFF files, refer to the Cadmus digital art website, <http://art.cadmus.com/da/index.jsp>. PowerPoint requires users to pay close attention to the fonts used in their images (see the section on fonts below). If

instructions for fonts are not followed exactly, images prepared for publication are subject to missing characters, improperly converted characters, or shifting/obscuring of elements or text in the figure. For proper font use in PowerPoint images, refer to the Cadmus digital art website, http://art.cadmus.com/da/instructions/ppt_disclaimer.jsp. Note that, due to page composition system requirements, you must verify that your PowerPoint files can be converted to PDF without any errors.

We strongly recommend that before returning their modified manuscripts, authors check the acceptability of their digital images for production by running their files through Rapid Inspector, a tool provided at the following URL: <http://rapidinspector.cadmus.com/RapidInspector/zmw/index.jsp>. Rapid Inspector is an easy-to-use, Web-based application that identifies file characteristics that may render the image unusable for production. Please note when using Rapid Inspector to check PowerPoint files that there is a known bug in the application that can occasionally fail PowerPoint Presentation (.pptx) files, even though the files meet all required production criteria. If you experience this bug, the issue can be corrected by saving the PowerPoint files as an older version, PowerPoint 97-2004 Presentation (.ppt), during the Save As process (use the drop-down format menu and select this format). Once you save your files as .ppt, they will pass Rapid Inspector if all required production criteria have been met.

If you have additional questions about using the Rapid Inspector preflighting tool, please send an e-mail inquiry to helpdesk.digitalsupport@cenveo.com.

Minimum resolution. It is extremely important that a high enough file resolution is used. All separate images that you import into a figure file must be at the correct resolution before they are placed. (For instance, placing a 72-dpi image in a 300-dpi EPS file will not result in the placed image meeting the minimum requirements for file resolution.) Note, however, that the higher the resolution, the larger the file and the longer the upload time. Publication quality will not be improved by using a resolution higher than the minimum. Minimum resolutions are as follows:

- 300 dpi for grayscale and color
- 600 dpi for combination art (lettering and images)
- 1,200 dpi for line art

Size. All graphics should be submitted at their intended publication size so that no reduction or enlargement is necessary. Resolution must be at the required level at the submitted size. Include only the significant portion of an illustration. White space must be cropped from the image, and excess space between panel labels and the image must be eliminated.

- Maximum figure width: 6.875 inches (ca. 17.4 cm)
- Maximum figure height: 9.0625 inches (23.0 cm)

Contrast. Illustrations must contain sufficient contrast to be viewed easily on a monitor or on the printed page.

Labeling and assembly. All final lettering and labeling must be incorporated into the figures. On initial submission, illustrations should be provided as PDF files, with the legends in the text file and with a legend beneath each image to assist review. At the modification stage, production-quality digital figure files (without legends) must be provided. Put the figure number well outside the boundaries of the image itself. (Numbering may need to be changed at the copyediting stage.) Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file; i.e., rather than uploading a separate file for each panel in a figure, assemble all panels in one piece and supply them as one file.

Fonts. To avoid font problems, set all type in one of the following fonts: Arial, Helvetica, Times Roman, European PI, Mathematical PI, or Symbol. Courier may be used but should be limited to nucleotide or amino acid sequences in which a nonproportional (monospace) font is required. All fonts other than these must be converted to paths (or outlines) in the application with which they were created.

Color illustrations. All figures submitted in color will be processed as color. Adherence to the following guidelines will help to ensure color reproduction that is as accurate as possible.

The final online version is considered the version of record for AAC and all other ASM journals. To maximize online reproduction, color illustrations should be supplied in the RGB color mode as either (i) RGB TIFF images with a resolution of at least 300 pixels per inch (raster files, consisting of pixels) or (ii) Illustrator-compatible EPS files with RGB color elements (vector files, consisting of lines, fonts, fills, and images). CMYK files are also accepted. Other than in color space, CMYK files must meet the same production criteria as RGB files. The RGB color space is the native color space of computer monitors and of most of the equipment and software used to capture scientific data, and it can display a wider range of colors (especially bright fluorescent hues) than the CMYK (cyan, magenta, yellow, black) color space used by print devices that put ink (or toner) on paper. For reprints, ASM's print provider will automatically create CMYK versions of color illustrations from the supplied RGB versions. Color in the reprints may not match that in the online journal of record because of the smaller range of colors capable of being reproduced by CMYK inks on a printing press. For additional information on RGB versus CMYK color, refer to the Cadmus digital art site, http://art.cadmus.com/da/guidelines_rgb.jsp.

Drawings. Submit graphs, charts, complicated chemical or mathematical formulas, diagrams, and other drawings as finished products not requiring additional artwork or typesetting. All elements, including letters, numbers, and symbols, must be easily readable, and both axes of a graph must be labeled.

When creating line art, please use the following guidelines:

(i) **All art must be submitted at its intended publication size.** For acceptable dimensions, see "Size," above.

(ii) **Avoid using screens (i.e., shading) in line art.** It can be difficult and time-consuming to reproduce these images without moiré patterns. Various pattern backgrounds are preferable to screens as long as the patterns are not imported from another application. If you must use images containing screens,

(a) Generate the image at line screens of 85 lines per inch or less.

(b) When applying multiple shades of gray, differentiate the gray levels by at least 20%.

(c) Never use levels of gray below 5% or above 95% as they are likely to fade out or become totally black when output.

(iii) Use thick, solid lines that are no finer than 1 point in thickness.

(iv) Use type that is no smaller than 6 points at the final publication size.

(v) Avoid layering type directly over shaded or textured areas.

(vi) Avoid the use of reversed type (white lettering on a black background).

(vii) Avoid heavy letters, which tend to close up, and unusual symbols, which the printer may not be able to reproduce in the legend.

(viii) If colors are used, avoid using similar shades of the same color and avoid very light colors.

In figure ordinate and abscissa scales (as well as table column headings), avoid the ambiguous use of numbers with exponents. Usually, it is preferable to use the Système International d'Unités (SI) symbols (μ for 10^{-6} , m for 10^{-3} , k for 10^3 , and M for 10^6 , etc.). Thus, a representation of 20,000 cpm on a figure ordinate should be made by the number 20 accompanied by the label kcpm. A complete listing of SI symbols can be found in the International Union of Pure and Applied Chemistry (IUPAC) publication *Quantities, Units and Symbols in Physical Chemistry*, 3rd ed. (RSC Publishing, Cambridge, United Kingdom, 2007), and at <https://www.nist.gov/physical-measurement-laboratory/special-publication-811>.

When powers of 10 must be used, the journal requires that the exponent power be associated with the number shown. In representing 20,000 cells per ml, the numeral on the ordinate should be “2” and the label should be “ 10^4 cells per ml” (not “cells per ml $\times 10^{-4}$ ”). Likewise, an enzyme activity of 0.06 U/ml might be shown as 6 accompanied by the label “ 10^{-2} U/ml.” The preferred designation is 60 mU/ml (milliunits per milliliter).

Presentation of Nucleic Acid Sequences

Long nucleic acid sequences must be presented as figures in the following format to conserve space. Print the se-

quence in lines of approximately 100 to 120 nucleotides in a nonproportional (monospace) font that is easily legible when published with a line length of 6 inches (ca. 15.2 cm). If possible, lines of nucleic acid sequence should be further subdivided into blocks of 10 or 20 nucleotides by spaces within the sequence or by marks above it. Uppercase and lowercase letters may be used to designate the exon-intron structure or transcribed regions, etc., if the lowercase letters remain legible at a 6-inch (ca. 15.2-cm) line length. Number the sequence line by line; place numerals representing the first base of each line to the left of the lines. Minimize spacing between lines of sequence, leaving room only for annotation of the sequence. Annotation may include boldface, underlining, brackets, and boxes, etc. Encoded amino acid sequences may be presented, if necessary, immediately above or below the first nucleotide of each codon, by using the single-letter amino acid symbols. Comparisons of multiple nucleic acid sequences should conform as nearly as possible to the same format.

Figure Legends

On initial submission, each legend should be placed in the text file *and* be incorporated into the image file beneath the figure to assist review.

Legends should provide enough information so that the figure is understandable without frequent reference to the text. However, detailed experimental methods must be described in the Materials and Methods section, not in a figure legend. A method that is unique to one of several experiments may be set forth in a legend only if the description is very brief (one or two sentences). Define all symbols used in the figure and define all abbreviations that are not used in the text.

Tables

Tables that contain artwork, chemical structures, or complex shading must be submitted as illustrations in an acceptable format at the modification stage. The preferred format for regular tables is Microsoft Word; however, WordPerfect and Acrobat PDF are also acceptable. Note that a straight Excel file is not currently an acceptable format. Excel files must be either embedded in a Word or WordPerfect document or converted to PDF before being uploaded.

Tables should be formatted as follows. Arrange the data so that **columns of like material read down, not across**. The headings should be sufficiently clear so that the meaning of the data is understandable without reference to the text. See the “**Abbreviations**” section of these Instructions for those that should be used in tables. Explanatory footnotes are acceptable, but more-extensive table “legends” are not. Footnotes should not include detailed descriptions of the experiment. Tables must include enough information to warrant table format; those with fewer than six pieces of data will be incorporated into the text by the copy editor. **Table 1** is an example of a well-constructed table.

Avoid tables (or figures) of raw data on drug susceptibility, therapeutic activity, or toxicity. Such data should be analyzed by an approved procedure, and the results should be presented in tabular form.

TABLE 1 Distribution of protein and ATPase in fractions of dialyzed membranes^a

Membrane	Fraction	ATPase	
		U/mg of protein	Total U
Control	Depleted membrane	0.036	2.3
	Concentrated supernatant	0.134	4.82
E1 treated	Depleted membrane	0.034	1.98
	Concentrated supernatant	0.11	4.6

^a Specific activities of ATPase of nondepleted membranes from control and treated bacteria were 0.21 and 0.20, respectively.

NOMENCLATURE

Chemical and Biochemical Nomenclature

The recognized authority for the names of chemical compounds is *Chemical Abstracts* (CAS; <http://www.cas.org/>) and its indexes. *The Merck Index Online* (<https://www.rsc.org/merck-index>) is also an excellent source. For guidelines to the use of biochemical terminology, consult *Biochemical Nomenclature and Related Documents* (Portland Press, London, United Kingdom, 1992), available at <http://www.sbcs.qmul.ac.uk/iupac/bibliog/white.html>, and the Instructions to Authors of the *Journal of Biological Chemistry*.

Molecular weight should not be expressed in daltons; molecular weight is a unitless ratio. Molecular mass is expressed in daltons.

For enzymes, use the recommended (trivial) name as assigned by the Nomenclature Committee of the International Union of Biochemistry (IUB) as described in *Enzyme Nomenclature* (Academic Press, Inc., New York, NY, 1992) and its supplements and at <http://www.sbcs.qmul.ac.uk/iubmb/enzyme/>. If a nonrecommended name is used, place the proper (trivial) name in parentheses at first use in the abstract and text. Use the EC number when one has been assigned. Authors of papers describing enzymological studies should review the standards of the STRENDA Commission for information required for adequate description of experimental conditions and for reporting enzyme activity data (<http://www.beilstein-institut.de/en/projects/strenda/guidelines>).

Nomenclature of Microorganisms

Binary names, consisting of a generic name and a specific epithet (e.g., *Escherichia coli*), must be used for all microorganisms. Names of categories at or above the genus level may be used alone, but specific and subspecific epithets may not. A specific epithet must be preceded by a generic name, written out in full the first time it is used in a paper. Thereafter, the generic name should be abbreviated to the initial capital letter (e.g., *E. coli*), provided there can be no confusion with other genera used in the paper. Names of all bacterial taxa (kingdoms, phyla, classes, orders, families, genera, species, and subspecies) are printed in italics and should be italicized in the manuscript; strain designations and numbers are not. Vernacular (common) names should be in lowercase roman type (e.g., streptococcus, brucella). For *Salmonella*, genus, species, and subspecies names should be rendered in standard form:

Salmonella enterica at first use, *S. enterica* thereafter; *Salmonella enterica* subsp. *arizonae* at first use, *S. enterica* subsp. *arizonae* thereafter. Names of serovars should be in roman type with the first letter capitalized: *Salmonella enterica* serovar Typhimurium. After the first use, the serovar may also be given without a species name: *Salmonella* Typhimurium, *S. Typhimurium*, or *Salmonella* serovar Typhimurium. For other information regarding serovar designations, see *Antigenic Formulae of the Salmonella Serovars*, 9th ed. (P. A. D. Grimont and F.-X. Weill, WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France, 2007; see <http://www.scacm.org/free/Antigenic%20Formulae%20of%20the%20Salmonella%20Serovars%202007%209th%20edition.pdf>). For a summary of the current standards for *Salmonella* nomenclature and the Kaufmann-White criteria, see the article by Brenner et al. (J Clin Microbiol 38:2465–2467, 2000), the opinion of the Judicial Commission of the International Committee on Systematics of Prokaryotes (Int J Syst Evol Microbiol 55:519–520, 2005), and the article by Tindall et al. (Int J Syst Evol Microbiol 55:521–524, 2005).

The spelling of bacterial names should follow the *Approved Lists of Bacterial Names (Amended) & Index of the Bacterial and Yeast Nomenclatural Changes* (V. B. D. Skerman et al., ed., American Society for Microbiology, Washington, DC, 1989) and the validation lists and notification lists published in the *International Journal of Systematic and Evolutionary Microbiology* (formerly the *International Journal of Systematic Bacteriology*) since January 1989. In addition, two sites on the World Wide Web list current approved bacterial names: Prokaryotic Nomenclature Up-to-Date (<https://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date.html>) and List of Prokaryotic Names with Standing in Nomenclature (<http://www.bacterio.net/>). If there is reason to use a name that does not have standing in nomenclature, the name should be enclosed in quotation marks in the title and at its first use in the abstract and the text and an appropriate statement concerning the nomenclatural status of the name should be made in the text. “*Candidatus*” species should always be set in quotation marks.

Since the classification of fungi is not complete, it is the responsibility of the author to determine the accepted binomial for a given organism. Sources for these names include *The Yeasts: a Taxonomic Study*, 5th ed. (C. P. Kurtzman, J. W. Fell, and T. Boekhout, ed., Elsevier Science, Amsterdam, Netherlands, 2011), and *Dictionary of the Fungi*, 10th ed. (P. M. Kirk, P. F. Cannon, D. W. Minter, and J. A. Stalpers, ed., CABI International, Wallingford, Oxfordshire, United Kingdom, 2008); see also <http://www.speciesfungorum.org/Names/Fundic.asp>.

Names used for viruses should be those approved by the International Committee on Taxonomy of Viruses (ICTV) and reported on the ICTV Virus Taxonomy website (<https://talk.ictvonline.org/>). In addition, the recommendations of the ICTV regarding the use of species names should generally be followed: when the entire species is discussed as a taxonomic entity, the species name, as with other taxa, is italic and has the first letter and any proper nouns capitalized (e.g., *Tobacco mosaic virus*, *Murray Valley encephalitis virus*). When the behavior or manipulation of individual viruses is discussed, the vernacular (e.g., tobacco mosaic virus, Murray Valley encephalitis virus) should be used. If desired, synonyms may be added parenthetically when the

name is first mentioned. Approved generic (or group) and family names may also be used.

Microorganisms, viruses, and plasmids should be given designations consisting of letters and serial numbers. It is generally advisable to include a worker's initials or a descriptive symbol of locale or laboratory, etc., in the designation. Each new strain, mutant, isolate, or derivative should be given a new (serial) designation. This designation should be distinct from those of the genotype and phenotype, and genotypic and phenotypic symbols should not be included. Plasmids are named with a lowercase "p" followed by the designation in uppercase letters and numbers. To avoid the use of the same designation as that of a widely used strain or plasmid, check the designation against a publication database such as Medline.

Genetic Nomenclature

To facilitate accurate communication, **it is important that standard genetic nomenclature be used whenever possible and that deviations or proposals for new naming systems be endorsed by an appropriate authoritative body.** Review and/or publication of submitted manuscripts that contain new or nonstandard nomenclature may be delayed by the editor or the Journals Department so that they may be reviewed.

Bacteria. The genetic properties of bacteria are described in terms of phenotypes and genotypes. The phenotype describes the observable properties of an organism. The genotype refers to the genetic constitution of an organism, usually in reference to some standard wild type. The guidelines that follow are based on the recommendations of Demerec et al. (Genetics 54:61–76, 1966).

(i) Phenotype designations must be used when mutant loci have not been identified or mapped. They can also be used to identify the protein product of a gene, e.g., the OmpA protein. Phenotype designations generally consist of three-letter symbols; these are not italicized, and the first letter of the symbol is capitalized. It is preferable to use Roman or Arabic numerals (instead of letters) to identify a series of related phenotypes. Thus, a series of nucleic acid polymerase mutants might be designated Pol1, Pol2, and Pol3, etc. Wild-type characteristics can be designated with a superscript plus (Pol^+), and, when necessary for clarity, negative superscripts (Pol^-) can be used to designate mutant characteristics. Lowercase superscript letters may be used to further delineate phenotypes (e.g., Str^r for streptomycin resistance). Phenotype designations should be defined.

(ii) Genotype designations are also indicated by three-letter locus symbols. In contrast to phenotype designations, these are lowercase italic (e.g., *ara his rps*). If several loci govern related functions, these are distinguished by italicized capital letters following the locus symbol (e.g., *araA araB araC*). Promoter, terminator, and operator sites should be indicated as described by Bachmann and Low (Microbiol Rev 44:1–56, 1980): e.g., *lacZp*, *lacAt*, and *lacZo*.

(iii) Wild-type alleles are indicated with a superscript plus (*ara⁺ his⁺*). A superscript minus is not used to indicate a mutant locus; thus, one refers to an *ara* mutant rather than an *ara⁻* strain.

(iv) Mutation sites are designated by placing serial isolation numbers (allele numbers) after the locus symbol (e.g., *araA1*

araA2). If only a single such locus exists or if it is not known in which of several related loci the mutation has occurred, a hyphen is used instead of the capital letter (e.g., *ara-23*). It is essential in papers reporting the isolation of new mutants that allele numbers be given to the mutations. For *Escherichia coli*, there is a registry of such numbers: *E. coli* Genetic Stock Center (<http://cgsc2.biology.yale.edu/>). For the genus *Salmonella*, the registry is *Salmonella* Genetic Stock Center (<http://people.ucalgary.ca/~kesander/>). For the genus *Bacillus*, the registry is *Bacillus* Genetic Stock Center (<http://www.bgsc.org/>).

(v) The use of superscripts with genotypes (other than + to indicate wild-type alleles) should be avoided. Designations indicating amber mutations (Am), temperature-sensitive mutations (Ts), constitutive mutations (Con), cold-sensitive mutations (Cs), production of a hybrid protein (Hyb), and other important phenotypic properties should follow the allele number [e.g., *araA230*(Am) *hisD21*(Ts)]. All other such designations of phenotype must be defined at the first occurrence. If superscripts must be used, they must be approved by the editor and defined at the first occurrence in the text.

Subscripts may be used in two situations. Subscripts may be used to distinguish between genes (having the same name) from different organisms or strains; e.g., *his_{E. coli}* or *his_{K-12}* for the *his* gene of *E. coli* or strain K-12, respectively, may be used to distinguish this gene from the *his* gene in another species or strain. An abbreviation may also be used if it is explained. Similarly, a subscript is also used to distinguish between genetic elements that have the same name. For example, the promoters of the *gln* operon can be designated *glnAp₁* and *glnAp₂*. This form departs slightly from that recommended by Bachmann and Low (e.g., *desC1p*).

(vi) Deletions are indicated by the symbol Δ placed before the deleted gene or region, e.g., $\Delta\text{trpA432}$, $\Delta(\text{aroP-aceE})419$, or $\Delta(\text{hisQ-hisJo})1256$. Similarly, other symbols can be used (with appropriate definition). Thus, a fusion of the *ara* and *lac* operons can be shown as $\Phi(\text{ara-lac})95$. Likewise, $\Phi(\text{araB'-lacZ}^+)96$ indicates that the fusion results in a truncated *araB* gene fused to an intact *lacZ* gene, and $\Phi(\text{malE-lacZ})97(\text{Hyb})$ shows that a hybrid protein is synthesized. An inversion is shown as $\text{IN}(\text{rrnD-rrnE})1$. An insertion of an *E. coli his* gene into plasmid pSC101 at zero kilobases (0 kb) is shown as pSC101 $\Omega(0\text{kb}::\text{K-12hisB})4$. An alternative designation of an insertion can be used in simple cases, e.g., *galT236::Tn5*. The number 236 refers to the locus of the insertion, and if the strain carries an additional *gal* mutation, it is listed separately. Additional examples, which utilize a slightly different format, can be found in the papers by Campbell et al. and Novick et al. cited below. It is important in reporting the construction of strains in which a mobile element was inserted and subsequently deleted that this fact be noted in the strain table. This can be done by listing the genotype of the strain used as an intermediate in a table footnote or by making a direct or parenthetical remark in the genotype, e.g., (F^-), $\Delta\text{Mu cts}$, or *mal::* $\Delta\text{Mu cts}::\text{lac}$. In setting parenthetical remarks within the genotype or dividing the genotype into constituent elements, parentheses and brackets are used without special meaning; brackets are used outside parentheses. To indicate the presence of an episome, parentheses (or brackets) are used (λ , F^+). Reference to an integrated episome is

indicated as described above for inserted elements, and an exogene is shown as, for example, W3110/F'8(*gal*⁺).

For information about genetic maps of locus symbols in current use, consult Berlyn (Microbiol Mol Biol Rev 62:814–984, 1998) for *E. coli* K-12, Sanderson and Roth (Microbiol Rev 52:485–532, 1988) for *Salmonella* serovar Typhimurium, Holloway et al. (Microbiol Rev 43:73–102, 1979) for the genus *Pseudomonas*, Piggot and Hoch (Microbiol Rev 49:158–179, 1985) for *Bacillus subtilis*, Perkins et al. (Microbiol Rev 46:426–570, 1982) for *Neurospora crassa*, and Mortimer and Schild (Microbiol Rev 49:181–213, 1985) for *Saccharomyces cerevisiae*. For yeasts, *Chlamydomonas* spp., and several fungal species, symbols such as those given in the *Handbook of Microbiology*, 2nd ed. (A. I. Laskin and H. A. Lechevalier, ed., CRC Press, Inc., Cleveland, OH, 1988) should be used.

Conventions for naming genes. It is recommended that (entirely) new genes be given names that are mnemonics of their function, avoiding names that are already assigned and earlier or alternative gene names, irrespective of the bacterium for which such assignments have been made. Similarly, it is recommended that, whenever possible, orthologous genes present in different organisms receive the same name. When homology is not apparent or the function of a new gene has not been established, a provisional name may be given by one of the following methods. (i) The gene may be named on the basis of its map location in the style *yaaA*, analogous to the style used for recording transposon insertions (*zef*) as discussed below. A list of such names in use for *E. coli* has been published by Rudd (Microbiol Mol Biol Rev 62:985–1019, 1998). (ii) A provisional name may be given in the style described by Demerec et al. (e.g., *usg*, gene upstream of *folC*). Such names should be unique, and names such as *orf* or *genX* should not be used. For reference, the *E. coli* Genetic Stock Center's database includes an updated listing of *E. coli* gene names and gene products. It is accessible on the Internet (<http://cgsc2.biology.yale.edu/index.php>). A list can also be found in the work of Riley (Microbiol Rev 57:862–952, 1993). For the genes of other bacteria, consult the references given above.

For prokaryotes, gene names should not begin with prefixes indicating the genus and species from which the gene is derived. (However, subscripts may be used where necessary to distinguish between genes from different organisms or strains as described in section v of “Bacteria,” above.) For eukaryotes, such prefixes may be used for clarity when discussing genes with the same name from two different organisms (e.g., *ScURA3* versus *CaURA3*); the prefixes are not considered part of the gene name proper and are not italicized.

Locus tags. Locus tags are systematic, unique identifiers that are assigned to each gene in GenBank. All genes mentioned in a manuscript should be traceable to their sequences by the reader, and locus tags may be used for this purpose in manuscripts to identify uncharacterized genes. In addition, authors should check GenBank to make sure that they are using the correct, up-to-date format for locus tags (e.g., uppercase versus lowercase letters and the presence or absence of an underscore, etc.). Locus tag formats vary between different organisms and also may be updated for a given organism, so it is important to check GenBank at the time of manuscript preparation.

“Mutant” versus “mutation.” Keep in mind the distinction between a mutation (an alteration of the primary sequence of the genetic material) and a mutant (a strain carrying one or more mutations). One may speak about the mapping of a mutation, but one cannot map a mutant. Likewise, a mutant has no genetic locus, only a phenotype.

“Homology” versus “similarity.” For use of terms that describe relationships between genes, consult the articles by Theissen (Nature 415:741, 2002) and Fitch (Trends Genet 16:227–231, 2000). “Homology” implies a relationship between genes that have a common evolutionary origin; partial homology is not recognized. When sequence comparisons are discussed, it is more appropriate to use the term “percent sequence similarity” or “percent sequence identity,” as appropriate.

Strain designations. Do not use a genotype as a name (e.g., “. . . subsequent use of *leuC6* for transduction . . .”). If a strain designation has not been chosen, select an appropriate word combination (e.g., “another strain containing the *leuC6* mutation”).

Viruses. The genetic nomenclature for viruses differs from that for bacteria. In most instances, viruses have no phenotype, since they have no metabolism outside host cells. Therefore, distinctions between phenotype and genotype cannot be made. Superscripts are used to indicate hybrid genomes. Genetic symbols may be one, two, or three letters. For example, a mutant strain of λ might be designated λ Aam11 *int2 red1* 14 cI857; this strain carries mutations in genes *cI*, *int*, and *red* and an amber-suppressible (Am) mutation in gene *A*. A strain designated λ att⁴³⁴ *imm*²¹ would represent a hybrid of phage λ that carries the immunity region (*imm*) of phage 21 and the attachment (*att*) region of phage 434. Host DNA insertions into viruses should be delineated by square brackets, and the genetic symbols and designations for such inserted DNA should conform to those used for the host genome. Genetic symbols for phage λ can be found in reports by Szybalski and Szybalski (Gene 7:217–270, 1979) and Echols and Murialdo (Microbiol Rev 42:577–591, 1978).

Eukaryotes. FlyBase (<http://flybase.org/>) is the genetic nomenclature authority for *Drosophila melanogaster*. WormBase (<http://www.wormbase.org/#01-23-6>) is the genetic nomenclature authority for *Caenorhabditis elegans*. When naming genes for *Aspergillus* species, the nomenclature guidelines posted at <http://www.aspergillusgenome.org/Nomenclature.shtml> should be followed, and the *Aspergillus* Genome Database (<http://www.aspgd.org/>) should be searched to ensure that any new name is not already in use. The *Saccharomyces* Genome Database (<http://www.yeastgenome.org/>) and the *Candida* Genome Database (<http://www.candidagenome.org/>) are authorities for *Saccharomyces cerevisiae* and *Candida albicans* genetic nomenclature, respectively. For information about the genetic nomenclature of other eukaryotes, see the Instructions to Authors for *Molecular and Cellular Biology*.

Transposable elements, plasmids, and restriction enzymes. Nomenclature of transposable elements (insertion sequences, transposons, and phage Mu, etc.) should follow the

recommendations of Campbell et al. (Gene 5:197–206, 1979), with the modifications given in section vi of “Bacteria,” above. The Internet site where insertion sequences of eubacteria and archaea are described and new sequences can be recorded is <https://www-is.biotoul.fr>.

The system of designating transposon insertions at sites where there are no known loci, e.g., *zef-123::Tn5*, has been described by Chumley et al. (Genetics 91:639–655, 1979). The nomenclature recommendations of Novick et al. (Bacteriol Rev 40:168–189, 1976) for plasmids and plasmid-specified activities, of Low (Bacteriol Rev 36:587–607, 1972) for F' factors, and of Roberts et al. (Nucleic Acids Res 31:1805–1812, 2003) for restriction enzymes, DNA methyltransferases, homing endonucleases, and their genes should be used whenever possible. The nomenclature for recombinant DNA molecules, constructed *in vitro*, follows the nomenclature for insertions in general. DNA inserted into recombinant DNA molecules should be described by using the gene symbols and conventions for the organism from which the DNA was obtained.

Tetracycline resistance determinants. The nomenclature for tetracycline resistance determinants is based on the proposal of Levy et al. (Antimicrob Agents Chemother 43:1523–1524, 1999). The style for such determinants is, e.g., Tet B; the space helps distinguish the determinant designation from that for phenotypes and proteins (TetB). The above-referenced article also gives the correct format for genes, proteins, and determinants in this family.

ABBREVIATIONS AND CONVENTIONS

Verb Tense

ASM strongly recommends that for clarity you use the **past** tense to narrate particular events in the past, including the procedures, observations, and data of the study that you are reporting. Use the present tense for your own general conclusions, the conclusions of previous researchers, and generally accepted facts. Thus, most of the abstract, Materials and Methods, and Results will be in the past tense, and most of the introduction and some of the Discussion will be in the present tense.

Be aware that it may be necessary to vary the tense in a single sentence. For example, it is correct to say “White (30) demonstrated that XYZ cells *grow* at pH 6.8,” “Figure 2 shows that ABC cells *failed* to grow at room temperature,” and “Air was removed from the chamber and the mice *died*, which *proves* that mice *require* air.” In reporting statistics and calculations, it is correct to say “The values for the ABC cells *are* statistically significant, indicating that the drug *inhibited* . . .”

For an in-depth discussion of tense in scientific writing, see *How To Write and Publish a Scientific Paper*, 7th ed.

Abbreviations

General. Abbreviations should be used as an aid to the reader, rather than as a convenience to the author, and therefore their **use should be limited**. Abbreviations other than those recommended by the IUPAC-IUB (*Biochemical Nomenclature and Related Documents*, 1992) should be used only when a case can be made for necessity, such as in tables and figures.

It is often possible to use pronouns or to paraphrase a long word after its first use (e.g., “the drug” or “the substrate”).

Standard chemical symbols and trivial names or their symbols (folate, Ala, and Leu, etc.) may also be used.

Define each abbreviation and introduce it in parentheses the first time it is used; e.g., “cultures were grown in Eagle minimal essential medium (MEM).” Generally, eliminate abbreviations that are not used at least three times in the text (including tables and figure legends).

Not requiring introduction. In addition to abbreviations for Système International d’Unités (SI) units of measurement, other common units (e.g., bp, kb, and Da), and chemical symbols for the elements, the following should be used without definition in the title, abstract, text, figure legends, and tables:

DNA (deoxyribonucleic acid)	reduced)
cDNA (complementary DNA)	NADP ⁺ (nicotinamide adenine dinucleotide phosphate, oxidized)
RNA (ribonucleic acid)	poly(A) and poly(dT), etc. (polyadenylic acid and polydeoxythymidylic acid, etc.)
cRNA (complementary RNA)	oligo(dT), etc. (oligodeoxythymidylic acid, etc.)
RNase (ribonuclease)	UV (ultraviolet)
DNase (deoxyribonuclease)	PFU (plaque-forming units)
rRNA (ribosomal RNA)	CFU (colony-forming units)
mRNA (messenger RNA)	MIC (minimal inhibitory concentration)
tRNA (transfer RNA)	Tris (tris[hydroxymethyl]aminomethane)
AMP, ADP, ATP, dAMP, ddATP, and GTP, etc. (for the respective 5' phosphates of adenosine and other nucleosides) (add 2', 3', or 5' - when needed for contrast)	DEAE (diethylaminoethyl)
ATPase and dGTPase, etc. (adenosine triphosphatase and deoxyguanosine triphosphatase, etc.)	EDTA (ethylenediamine-tetraacetic acid)
NAD (nicotinamide adenine dinucleotide)	EGTA (ethylene glycol-bis[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid)
NAD ⁺ (nicotinamide adenine dinucleotide, oxidized)	HEPES (N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid)
NADH (nicotinamide adenine dinucleotide, reduced)	PCR (polymerase chain reaction)
NADP (nicotinamide adenine dinucleotide phosphate)	AIDS (acquired immunodeficiency syndrome)
NADPH (nicotinamide adenine dinucleotide phosphate,	

Abbreviations for cell lines (e.g., HeLa) also need not be defined.

The following abbreviations should be used without definition in tables:

amt (amount)	SD (standard deviation)
approx (approximately)	SE (standard error)
avg (average)	SEM (standard error of the mean)
concn (concentration)	sp act (specific activity)
diam (diameter)	sp gr (specific gravity)
expt (experiment)	temp (temperature)
exptl (experimental)	vol (volume)
ht (height)	vs (versus)
mo (month)	wk (week)
mol wt (molecular weight)	wt (weight)
no. (number)	yr (year)
prepn (preparation)	

Drugs and pharmaceutical agents. Should an author decide to abbreviate the names of antimicrobial agents in a man-

uscript, the following standard abbreviations are strongly recommended.

(i) Antibacterial agents. Use the indicated abbreviations for the following antibacterial agents.

amikacin (AMK)	enoxacin (ENX)
amoxicillin (AMX)	erythromycin (ERY)
amoxicillin-clavulanic acid (AMC)	floxacin (FLE)
ampicillin (AMP)	fosfomicin (FOF)
ampicillin-sulbactam (SAM)	gatifloxacin (GAT)
azithromycin (AZM)	gentamicin (GEN)
azlocillin (AZL)	grepafloxacin (GRX)
aztreonam (ATM)	imipenem (IPM)
carbenicillin (CAR)	kanamycin (KAN)
cefaclor (CEC)	levofloxacin (LVX)
cefadroxil (CFR)	linezolid (LZD)
cefamandole (FAM)	lomefloxacin (LOM)
cefazolin (CFZ)	loracarbef (LOR)
cefdinir (CDR)	meropenem (MEM)
cefditoren (CDN)	methicillin (MET)
cefepime (FEP)	mezlocillin (MEZ)
cefetamet (FET)	minocycline (MIN)
cefixime (CFM)	moxalactam (MOX)
ceftazidime (CAZ)	moxifloxacin (MXF)
ceftazidime-avibactam (CZA)	nafcillin (NAF)
ceftibuten (CTB)	nalidixic acid (NAL)
ceftizoxime (ZOX)	netilmicin (NET)
ceftolozane-tazobactam (C/T)	nitrofurantoin (NIT)
ceftriaxone (CRO)	norfloxacin (NOR)
cefuroxime (axetil or sodium) (CXM)	ofloxacin (OFX)
cephalexin (LEX)	oritavancin (ORI)
cephalothin (CEF)	oxacillin (OXA)
cephapirin (HAP)	penicillin (PEN)
cephradine (RAD)	piperacillin (PIP)
chloramphenicol (CHL)	piperacillin-tazobactam (TZP)
cinoxacin (CIN)	polymyxin B (PMB)
ciprofloxacin (CIP)	quinupristin-dalfopristin (Synercid) (Q-D)
clarithromycin (CLR)	rifabutin (RFB)
clinafloxacin (CLX)	rifampin (RIF)
clindamycin (CLI)	rifapentine (RFP)
colistin (CST)	sparfloxacin (SPX)
dalbavancin (DAL)	spectinomycin (SPT)
daptomycin (DAP)	streptomycin (STR)
dicloxacillin (DCX)	tedizolid (TZD)
dirithromycin (DTM)	teicoplanin (TEC)
doripenem (DOR)	telavancin (TLV)
doxycycline (DOX)	telithromycin (TEL)
	tetracycline (TET)
	ticarcillin (TIC)
	ticarcillin-clavulanic acid (TIM)
	tigecycline (TGC)
	tobramycin (TOB)
	trimethoprim (TMP)
	trimethoprim-sulfamethoxazole (SXT)
	trovafloxacin (TVA)
	vancomycin (VAN)

(ii) β -Lactamase inhibitors. Use the indicated abbreviations for the following β -lactamase inhibitors.

avibactam (AVI)	sulbactam (SUL)
clavulanic acid (CLA)	tazobactam (TZB)

(iii) Antifungal agents. Use the indicated abbreviations for the following antifungal agents.

amphotericin B (AMB)	itraconazole (ITC)
anidulafungin (AFG)	ketoconazole (KTC)
caspofungin (CAS)	miconazole (MFG)
clotrimazole (CLT)	nystatin (NYT)
flucytosine (5FC)	posaconazole (POS)
fluconazole (FLC)	terbinafine (TRB)
isavuconazole (ISA)	voriconazole (VRC)

(iv) Antiviral agents. Use the indicated abbreviations for the following antiviral agents (for additional agents, refer to Table 2 in De Clercq and Li, *Clin Microbiol Rev* 29:695–747, 2016 [<https://doi.org/10.1128/CMR.00102-15>]).

acyclovir (ACV)	ganciclovir (GCV)
cidofovir (CDV)	penciclovir (PCV)
famciclovir (FCV)	valacyclovir (VCV)
foscarnet (FOS)	zidovudine (AZT)

(v) Antimycobacterial agents. Use the indicated abbreviations for the following antimycobacterial agents.

bedaquiline (BDQ)	ethionamide (ETO)
capreomycin (CAP)	isoniazid (INH)
clofazimine (CLO)	<i>para</i> -aminosalicylic acid (PAS)
D-cycloserine (DCS)	prothionamide (PTO)
delamanid (DMD)	pyrazinamide (PZA)
ethambutol (EMB)	

The use of “nonstandard” abbreviations to designate names of antibiotics and other pharmaceutical agents generally will not be accepted, because the use of different abbreviations for a single agent has often caused confusion. If, on occasion, a nonstandardized abbreviation for a drug or pharmaceutical substance is used, it will be accepted under the following conditions: (i) it must be defined at the first use in the text, (ii) it must be unambiguous in meaning, and (iii) it must contribute to ease of assimilation by readers.

Chemical or generic names of drugs should be used; the use of trade names is not permitted. Avoid the ambiguous term “generation” when classes of drugs are described. When code names or corporate proprietary numbers are to be used, either the chemical structure of the compound or a published literature reference illustrating the chemical structure, if known, must be provided at the first occurrence of the code name or number. For compounds not identified by generic nomenclature, all previous or concurrent identification numbers or appellations should be listed in the manuscript.

Pharmacodynamic terminology. Pharmacodynamic indices (PDIs) must be introduced at their first occurrence in the text and follow guidelines set forth by Mouton et al. (*J Antimicrob Chemother* 55:601–607, 2005). In Materials and Methods, it should be clearly stated how the PDIs were derived. The most common indices used are the following: AUC/MIC ratio (the area under the concentration-time curve over 24 h in steady state divided by the MIC), AUIC (the area under the inhibitory curve; note that the AUC/MIC ratio is not

equal to the AUC), $\%T_{MIC}$ (the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions), C_{max}/MIC ratio (the peak level divided by the MIC), PTA (probability of target attainment), and CFR (cumulative fraction of response). Clear distinction should be made between $\%T_{MIC}$, which is expressed as a percentage of the dosing interval, and T_{MIC} , expressed in hours. It is strongly recommended that the prefix *f* be used with an index (e.g., *fAUC*) if the free, unbound fraction of the drug is meant.

β -Lactamases

Studies performed to characterize a β -lactamase or the interaction of a compound with a β -lactamase (i.e., as a substrate, inhibitor, or inducer) should follow the guidelines set forth by Bush and Sykes (*Antimicrob Agents Chemother* 30:6–10, 1986). Assays that measure the hydrolysis of β -lactam antibiotics must be appropriate for the substrate examined (e.g., iodometric methods are not appropriate quantitative assays for substrates whose products are unknown). Reproducibility of results must be shown. When referring to β -lactamases, please use the functional designations defined by Bush and Jacoby (*Antimicrob Agents Chemother* 54:969–976, 2010). Alternatively, if the amino acid sequence for the enzyme is known, the β -lactamases may be described by molecular class as initiated by Ambler (*Philos Trans R Soc Lond B Biol Sci* 289:321–331, 1980).

A database of defining amino acid alterations for many β -lactamases is maintained at https://www.ncbi.nlm.nih.gov/projects/pathogens/submit_beta_lactamase/. The managers of that site should be consulted about the name of a potentially novel β -lactamase sequence before a new designation or number is proposed for publication.

In Vitro Susceptibility Tests

Tabulate results of determinations of minimal inhibitory and bactericidal concentrations according to the range of concentrations of each antimicrobial agent required to inhibit or kill the members of a species or of each group of microorganisms tested, as well as the corresponding concentrations required to inhibit 50 and 90% of the strains (MIC_{50} and MIC_{90} , respectively) and those required to kill 50 and 90% of the strains (MBC_{50} and MBC_{90} , respectively). Geometric mean MICs may also be reported when relevant. The MIC_{50} and MIC_{90} reported should be the actual concentrations tested that inhibited 50 and 90%, respectively, of the strains. They should not be values calculated from the actual data obtained. When only six to nine isolates of a species are tested, tabulate only the MIC range of each antimicrobial agent tested.

If more than a single drug is studied, insert a column labeled “Test agent” between the columns listing the organisms and the columns containing the numerical data and record data for each agent in the same isolate order. Cumulative displays of MICs or MBCs in tables or figures are acceptable only under unusual circumstances.

The percentage of strains susceptible and/or resistant to an antibiotic at its breakpoint concentration may be given only if an appropriate breakpoint has been approved, as by the Clin-

ical and Laboratory Standards Institute (<https://clsi.org/>). In the absence of approved breakpoints, authors cannot assign breakpoints or use breakpoints from related antibiotics. An exploratory analysis tabulating the percentage of strains inhibited over a range of concentrations is acceptable.

Bactericidal tests must be performed with a sufficient inoculum ($>5 \times 10^5$ CFU/ml) and subculture volume (0.01 ml) to ensure accurate determination of the 99.9% killing endpoint, as described by Pearson et al. (*Antimicrob Agents Chemother* 18:699–708, 1980) and Taylor et al. (*Antimicrob Agents Chemother* 23:142–150, 1983). Inoculum size and subculture volume are also critical to studies of combinations of antimicrobial agents.

Synergy is defined in two-dimensional or checkerboard tests when the fractional inhibitory concentration (FIC) or fractional bactericidal concentration (FBC) index (Σ) is ≤ 0.5 . In killing curves, synergy is defined as a ≥ 2 -log₁₀ decrease in CFU per milliliter between the combination and its most active constituent after 24 h, and the number of surviving organisms in the presence of the combination must be ≥ 2 log₁₀ CFU/ml below the starting inoculum. At least one of the drugs must be present in a concentration which does not affect the growth curve of the test organism when used alone. Antagonism is defined by a Σ FIC or Σ FBC of >4.0 .

When standard twofold-dilution schemes are used to determine checkerboard interactions, the inherent variability of the method casts doubt on the significance of interactions represented by Σ FICs or Σ FBCs of >0.5 but ≤ 4 . Therefore, such interactions, if labeled at all, should be termed “indifferent.” Alternatively, indices in this range may be described as “nonsynergistic” or “nonantagonistic,” as appropriate. The technically imprecise term “additive” should be avoided, as it is too easily misunderstood. See reports by W. R. Greco et al. (*Pharmacol Rev* 47:331–385, 1995), F. C. Odds (*J Antimicrob Chemother* 52:1, 2003), and M. D. Johnson et al. (*Antimicrob Agents Chemother* 48:693–715, 2004) for further discussion of these issues.

For killing curve tests, the minimal, accurately countable number of CFU per milliliter must be stated and the method used for determining this number must be described. In the absence of any drug and with a sample size of 1 ml, this number is 30 (1.5 in log₁₀) CFU. If procedures for drug inactivation or removal have not been performed, the author must state how drug carryover effects were eliminated or quantified. For drugs showing an inoculum effect, mere dilution below the MIC obtained in standard tests is not sufficient.

Clinical Trials

(i) Registration. AAC requires the prospective registration (i.e., before the first patient is enrolled) of a clinical trial in a public trials registry in accordance with guidelines established by the International Committee of Medical Journal Editors (ICMJE) (<http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html>). The ICMJE defines a clinical trial as “any research project that prospectively assigns people or a group of people to an intervention, with or without concurrent comparison or control groups, to study the cause-and-effect relationship between a health-related intervention *and* a health outcome.”

AAC does not require registration in a particular registry, but the registry chosen must meet the following criteria, in agreement with ICMJE recommendations. It must be (a) accessible to the public free of charge, (b) open to all registrants, (c) managed by a not-for-profit organization, (d) monitored by a mechanism to ensure validity of registration data, and (e) searchable electronically. A registration with missing fields or uninformative terminology will be deemed inadequate.

The registry and the trial registration number must be included at the end of the abstract. If a registration number is available, the authors should state this number the first time a trial acronym is used to refer to the trial being reported or to other trials mentioned in the manuscript.

(ii) Criteria for enrollment. The methods used to find and enroll patients and the criteria for enrollment in a clinical trial should be stated. In addition, the time period (month/year to month/year) of the enrollment should be specified. It should be indicated, if appropriate, that written informed consent was obtained and that the trial was approved by the pertinent committee on human subjects.

(iii) Method of randomization. Randomized, double-blind studies are preferred. Comparisons using historical controls are usually regarded as questionable unless the differences in outcome between the groups are dramatic and almost certainly the result of the new intervention. The rationale for the choice of the control group should be explained. The sample size should be justified, and the method of randomization should be stated.

(iv) Criteria for determining whether a case is evaluable. The minimum criteria for evaluability should be stated explicitly. For example, it should be stated that the minimum criterion for evaluability was *a* or the combination of *b* and *c* rather than *a*, *b*, and *c* without designating which were the minimum criteria. The criteria for evaluability are usually different from those for enrollment.

(v) Reasons for nonevaluability. State the number of patients in each group who were excluded from evaluation and the reason(s) for each exclusion.

(vi) Criteria for assessment. Define each outcome for each category of assessment (e.g., “clinical outcomes were classified as cure, improvement, and failure; microbiological outcomes were classified as eradication, persistence, and relapse”). The frequency and timing of such assessments in relation to treatment should be stated. Specify any changes made in the study regimen(s) during the trial; the results for regimens with and without such modification generally should be stated separately. The criteria (questionnaires, results of specific laboratory tests) for evaluation of adverse effects should be stated, as should the period encompassed in the assessment and the time of assessment in relation to the time of treatment (e.g., daily during treatment). Some authors prefer to consider superinfections as failures of treatment, whereas others prefer to consider them separately or even as adverse effects. In any event, the manuscript should state the number of superinfec-

tions with each regimen and should differentiate between superinfections and colonization. The duration of follow-up should be mentioned.

(vii) Statistical analyses. The type of statistical test should be stated, and when appropriate, the reason for the choice of test should be given. References should be given for statistical procedures other than the *t* test, chi-square test, and Wilcoxon rank sum test. The comparability of the treatment groups at the baseline should be evaluated statistically.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the articles by Olsen (Infect Immun 71:6689–6692, 2003; Infect Immun 82:916–920, 2014).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J Virol 79:669–676, 2005).

(viii) Beta error. For trials which show no statistically significant difference between regimens, calculate the probability (β) of a type II error and the power of the study ($1 - \beta$) to detect a specified clinically meaningful difference in efficacy between the regimens. For further details, see the article by Freiman et al. (N Engl J Med 299:690–694, 1978). Alternatively, or in addition, indicate the magnitude of difference between the regimens that could have been detected at a statistically significant level with the number of evaluable patients studied.

For further details, see the editorial on guidelines for clinical trials (Antimicrob Agents Chemother 33:1829–1830, 1989).

Reporting Numerical Data

Standard metric units are used for reporting length, weight, and volume. For these units and for molarity, use the prefixes m, μ , n, and p for 10^{-3} , 10^{-6} , 10^{-9} , and 10^{-12} , respectively. Likewise, use the prefix k for 10^3 . Avoid compound prefixes such as m μ or $\mu\mu$. Use $\mu\text{g/ml}$ or $\mu\text{g/g}$ in place of the ambiguous ppm. Units of temperature are presented as follows: 37°C or 324 K.

When fractions are used to express units such as enzymatic activities, it is preferable to use whole units, such as g or min, in the denominator instead of fractional or multiple units, such as μg or 10 min. For example, “pmol/min” is preferable to “nmol/10 min,” and “ $\mu\text{mol/g}$ ” is preferable to “nmol/ μg .” It is also preferable that an unambiguous form, such as exponential notation, be used; for example, “ $\mu\text{mol g}^{-1} \text{min}^{-1}$ ” is preferable to “ $\mu\text{mol/g/min}$.” Always report numerical data in the appropriate SI units.

Representation of data as accurate to more than two significant figures must be justified by presentation of appropriate statistical analyses.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the articles by Olsen (Infect Immun 71:6689–6692, 2003; Infect Immun 82:916–920, 2014).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J Virol 79:669–676, 2005).

Isotopically Labeled Compounds

For simple molecules, labeling is indicated in the chemical formula (e.g., $^{14}\text{CO}_2$, $^3\text{H}_2\text{O}$, and $\text{H}_2^{35}\text{SO}_4$). Brackets are not used when the isotopic symbol is attached to the name of a compound that in its natural state does not contain the element (e.g., ^{32}S -ATP) or to a word that is not a specific chemical name (e.g., ^{131}I -labeled protein, ^{14}C -amino acids, and ^3H -ligands).

For specific chemicals, the symbol for the isotope introduced is placed in square brackets directly preceding the part of the name that describes the labeled entity. Note that configuration symbols and modifiers precede the isotopic symbol. The following examples illustrate correct usage:

^{14}C urea	$[\gamma\text{-}^{32}\text{P}]\text{ATP}$
L-[<i>methyl</i> - ^{14}C]methionine	UDP-[U- ^{14}C]glucose
[2,3- ^3H]serine	<i>E. coli</i> [^{32}P]DNA
$[\alpha\text{-}^{14}\text{C}]\text{lysine}$	fructose 1,6-[1- ^{32}P]bisphosphate