



FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2013/2014

João Guilherme Neves Maia

Species distribution and in vitro antifungal
susceptibility profile of yeast isolates from invasive
infections on a Portuguese multicenter survey

março, 2014

FMUP



FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

João Guilherme Neves Maia
Species distribution and in vitro
antifungal susceptibility profile of yeast
isolates from invasive infections on a
Portuguese multicenter survey.

Mestrado Integrado em Medicina

Área: Microbiologia Clínica

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

**Trabalho organizado de acordo com as normas da revista:
Journal of Clinical Microbiology**

março, 2014

FMUP

Eu, João Guilherme Neves Maia, abaixo assinado, nº mecanográfico 200801966, 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.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 20/03/2014

Assinatura conforme cartão de identificação:

João Guilherme Neves Maia

NOME

João Guilherme Neves Maia

CARTÃO DE CIDADÃO OU PASSAPORTE (se estrangeiro)

E-MAIL

TELEFONE OU TELEMÓVEL

13503655

joagnmaia@gmail.com

915487568

NÚMERO DE ESTUDANTE

DATA DE CONCLUSÃO

200801966

20/03/2014

DESIGNAÇÃO DA ÁREA DO PROJECTO

Microbiologia Clínica

TÍTULO DISSERTAÇÃO/MONOGRAFIA (riscar o que não interessa)

Species distribution and in vitro antifungal susceptibility profile of yeast isolates from invasive infections on a Portuguese multicenter survey.

ORIENTADOR

Professora Doutora Cidália Pina Vaz

COORIENTADOR (se aplicável)

É autorizada a reprodução integral desta Dissertação/Monografia (riscar o que não interessa) para efeitos de investigação e de divulgação pedagógica, em programas e projectos coordenados pela FMUP.

Faculdade de Medicina da Universidade do Porto, 20/03/2014

Assinatura conforme cartão de identificação: João Guilherme Neves Maia

1 **Species distribution and *in vitro* antifungal susceptibility profile of yeast**
2 **isolates from invasive infections on a Portuguese multicenter survey.**

3

4 I. Faria-Ramos,^a J. Neves-Maia,^a E. Ricardo,^a J. Santos-Antunes,^{a,b} A. T. Silva,^{a,c,d} S.
5 Costa-de-Oliveira,^{a,c,d} E. Cantón,^e A. G. Rodrigues,^{a,c,d,f} C. Pina-Vaz,^{a,c,d,g,#}

6

7 Microbiology Department, Faculty of Medicine, University of Porto, Porto, Portugal^a;
8 Gastroenterology Department, Faculty of Medicine, Centro Hospitalar de São João,
9 Porto, Portugal^b; Cardiovascular Research & Development Unit, Faculty of Medicine,
10 University of Porto, Portugal^c; CINTESIS - Center for Health Technology and Services
11 Research, Faculty of Medicine of the University of Porto, Portugal^d; Unidad de
12 Microbiología Experimental, Centro de Investigación, Hospital La Fe, Valencia, Spain^e;
13 Burn Unit, Centro Hospitalar de São João, Porto, Portugal^f; Microbiology Department,
14 Centro Hospitalar de São João, Porto, Portugal^g

15

16 Running Head: Fungemia in Portugal

17 # Address correspondence to Cidália Pina-Vaz, Microbiology Department, Faculty of
18 Medicine, University of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto,
19 Portugal (cpinavaz@yahoo.com)

20

21

22 ABSTRACT

23 Invasive yeast infections (IYI) represent a serious nosocomial problem. This is
24 the first Portuguese multicenter prospective, observational and descriptive study that
25 provides insights on species distribution and susceptibility profile of yeast isolates from
26 fungemia episodes. All Portuguese hospitals were invited to integrate this study during
27 2012 (a twelve-month period). Ten district hospitals across mainland Portugal
28 contributed by collecting yeast isolates from blood cultures and answering
29 questionnaires concerning patients' data. The susceptibility profile of each isolate,
30 considering eight of the most used antifungals, was determined. Two hundred and
31 forty yeast isolates were recovered and identified. Incidence of fungemia was
32 0.88/1000 admissions. Fifteen different species were found, with *Candida albicans* as
33 the most prevalent; however, 60% of fungemias were caused by non-*albicans* species.
34 Most isolates were recovered from patients admitted at surgical wards or Intensive
35 Care Units with 57% being males and 32% aged 41 to 60 years old. About 13% of yeast
36 isolates were resistant to fluconazole, 18% to posaconazole and 16% to voriconazole.
37 Regarding echinocandins, the highest percentage of resistance was to caspofungin
38 (17%). Rare cases of strains displaying high MIC values to amphotericin B were also
39 obtained (n=3). Death within 30 days associated with IYI occurred in 25% of the cases
40 with more than half of *C. glabrata* infections being fatal. Studies addressing species
41 distribution and susceptibility profile are extremely valuable as they provide important
42 clues about worldwide IYI tendencies and may help improving empirical treatment
43 guidelines.

44

45 INTRODUCTION

46 Increased incidence of invasive yeast infections (IYI) represents a clinical
47 problem and its impact has significantly risen during the last twenty years (1).
48 Fungemia is an important cause of morbidity and mortality, being related to longer
49 hospital stays and very high economic costs. *Candida albicans* still remains as the
50 leading cause of fungemia worldwide (2). *Candida parapsilosis*, *Candida glabrata* and
51 *Candida tropicalis* occupy the following places, varying according to the region. Due to
52 the medical relevance of IYI and its very strong association with an unfavorable
53 outcome, epidemiological surveillance studies are urgently needed in order to evaluate
54 species' geographic distribution and changes in susceptibility profiles. In addition,
55 there is a limited number of therapeutic options, with the main class of drugs (azoles)
56 controversially used for prophylaxis, possibly leading to decreased susceptibility and
57 the selection of non-*albicans* species. In recent years we have witnessed several
58 worldwide epidemiological and clinical changes regarding IYI (3-5). The aim of this
59 study was to provide an overview about IYIs in Portugal and to evaluate the
60 susceptibility profile of yeasts isolated from blood cultures.

61

62 MATERIALS AND METHODS

63 **Study design** Ten hospitals from northern (four), central (two) and southern
64 (four) regions of Portugal accepted to participate in this study, providing isolates
65 collected from patients with fungemia from September 2011 to September 2012. Two
66 hospitals had more than 1000 beds, 3 had between 600 and 1000 beds and 5 had less
67 than 600 beds. All participating hospitals were asked to collect and send the strains to

68 the Microbiology Department of the Faculty of Medicine of the University of Porto,
69 where the study was conducted. In addition, a questionnaire regarding patient's
70 clinical and demographic data was also sent.

71 **Definitions** An episode of fungemia was defined as the first isolation from a
72 blood culture of a yeast strain from a patient with related signs and symptoms.
73 Nosocomial fungemia was defined whenever the yeast isolate was obtained more than
74 48h after hospital admission. Ages were grouped into five categories: less than 20
75 years, 20-40 years, 41-60 years, 61-70 years and more than 70 years old. For each
76 individual patient, the outcome of the fungemia episode was evaluated at 30 days
77 after the first yeast isolation. Death associated with fungemia was defined as death
78 within 30 days after recovery of the first yeast isolate, without any other concomitant
79 cause of death, like intracerebral or gastrointestinal bleeding or pulmonary embolism.

80 **Identification and in vitro antifungal susceptibility testing of yeast isolates**

81 Yeasts were identified using Vitek 2 YST card from bioMérieux (Paris, France). The
82 characterization of *Candida glabrata* sensu stricto, *Candida bracarensis* and *Candida*
83 *nivariensis* was confirmed as previously described by Romeo *et al* (6). *Candida*
84 *parapsilosis* sensu stricto, *Candida orthopsilosis* and *Candida metapsilosis* isolates were
85 identified as previously described by Tavanti *et al* (7). Furthermore, the identities of
86 these species were confirmed by DNA amplification and further sequencing of *ITS1* and
87 *ITS4* regions of rRNA genes. Minimal inhibitory concentrations (MIC) were determined
88 by broth microdilution, following the CLSI guidelines (8). Antifungal powders were
89 obtained from the manufacturers - fluconazole (FLC), voriconazole (VRC) and
90 anidulafungin (AND) [from Pfizer, Groton, C.T., USA], posaconazole (POS) [Schering-

91 Plough Research Institute, Kenilworth, N.J., USA], caspofungin (CAS) [from Merck,
92 Rahway, N.J., USA], micafungin (MCF) [from Astellas Pharma, UK], flucytosine (5FC)
93 [Roche Laboratory Inc., Nutley, N.J., USA] and deoxycholate amphotericin B (AMB)
94 [Bristol-Myers Squibb, N.Y., USA]. FLC, CAS and MCF were dissolved in water and the
95 other drugs in DMSO. The new species-specific breakpoints for FLC, VRC and
96 echinocandins were applied (8, 9). Isolates of *C. albicans*, *C. tropicalis* and *C.*
97 *parapsilosis* for which FLC MICs were ≤ 2 mg/L were categorized as susceptible and
98 resistant at MICs > 4 mg/L. *C. glabrata* was considered FLC susceptible-dose dependent
99 (S-DD) at MICs ≤ 32 mg/L and resistant at MICs > 32 mg/L. *C. albicans*, *C. tropicalis*, and
100 *C. parapsilosis* were classified as susceptible to VRC at MICs ≤ 0.125 mg/L, and as
101 resistant at ≥ 1 mg/L, and for *C. krusei*, susceptible when MICs ≤ 0.5 mg/L and resistant
102 if > 1 mg/L. Isolates of *C. albicans*, *C. tropicalis*, and *C. krusei*, for which AND, CAS and
103 MCF MICs were ≤ 0.25 mg/L, were classified as susceptible and > 0.5 mg/L as resistant.
104 *C. glabrata* was categorized as susceptible when AND or CAS MICs were ≤ 0.12 mg/L
105 and as resistant when > 0.25 mg/L, while for MCF they were ≤ 0.06 mg/L (susceptible)
106 and > 0.12 mg/L (resistant). *C. parapsilosis* and *Candida guilliermondii* were classified as
107 susceptible or resistant when MICs of the three echinocandins were ≤ 2 mg/L and > 4
108 mg/L, respectively. For POS and 5FC the epidemiological cut-off values were applied to
109 separate the wild type from non-wild type isolates: 0.06 mg/L for *C. albicans*, 0.12
110 mg/L for *C. tropicalis*, 0.25 mg/L for *Candida lusitanae* and *C. parapsilosis*, 0.5 mg/L for
111 *C. krusei* and 2 mg/L for *C. glabrata* for POS; and for 5FC it was 0.5 mg/L for *C. albicans*,
112 *C. tropicalis*, *C. glabrata* and *C. parapsilosis* and 32 mg/L for *C. krusei* (9-11). MICs were
113 registered after 24 and 48 hours for *Candida* species and after 48 and 72 hours for
114 *Cryptococcus neoformans*. Since breakpoints for AMB have not yet been established,

115 yeasts inhibited by ≤ 1 mg/L were considered to be susceptible according to
116 suggestions by Pfaller *et al* (11). The quality control (QC) strains *C. parapsilosis* ATCC
117 22019 and *C. krusei* ATCC 6258 were included in all assays (8).

118 **Statistical analysis** A descriptive revision of the collected data was performed
119 using the statistic program SPSS v21.0 (SPSS Software, Chicago, USA). The Chi-square
120 test was used to compare proportions and analyze differences in the species
121 distribution and in antifungal susceptibility profiles.

122

123 RESULTS

124 **Patient's data** A total of 240 fungemia episodes were reported in the current
125 survey (corresponding to 240 different patients) and included in the study. The mean
126 incidence of fungemia was 0.88 per 1000 admissions, ranging from 0.15 to 2.4. The
127 mean incidence of nosocomial fungemia was 0.74 per 1000 admissions, ranging from
128 0.14 to 2.1, and corresponding to approximately 86% of all episodes of fungemia. Fifty-
129 seven per cent of patients with fungemia were males and the most affected group was
130 the one aged 41-60 years old (32%) closely followed by the group of patients over 70,
131 together accounting for 62% of cases (Table1). Most patients enrolled in this study
132 were admitted at ICU (39%) and surgical wards (30%). The crude mortality rate
133 associated with fungemia was 25%. The deadliest species was *C. glabrata*, with more
134 than 50% of patients dying within 30 days of fungemia detection. The other main
135 *Candida* species did not differ much from the general value. (Table 1)

136

137 **Species distribution and identification** Fifteen different yeast species were
138 identified, with the five most prevalent being *C. albicans* (40.4%), *C. parapsilosis*
139 (22.9%), *C. glabrata* (13.3%), *C. tropicalis* (6.3%) and *C. krusei* (5%). *C. neoformans* was
140 responsible for eight (3.3%) episodes of fungemia followed by *C. lusitaniae* (2.5%),
141 *Candida guilliermondii* (1.7%), *Candida dubliniensis* (1.3%) and *Candida famata* (1.3%).
142 Single cases due to *Candida sake*, *Candida inconspicua*, *Candida haemulloni*, *Candida*
143 *kefyr* and *Trichosporon mucoides* were also found, as a whole accounting for 1.7% of
144 all cases. Regarding the molecular identification of the cryptic species, from 55 isolates
145 of *C. parapsilosis* sensu lato, 49 corresponded to *C. parapsilosis* sensu stricto, 4 to *C.*
146 *orthopsilosis* (3 isolated from Medicine Department and 1 from UCI; 1 belonging to a
147 52 years old and the other 3 to patients over 70 years old) and 2 to *C. metapsilosis*
148 (both from the Medicine Department; one from a 17 year old patient and the other
149 from a 32 year-old patient). The 32 isolates primarily identified as belonging to the *C.*
150 *glabrata* group corresponded to *C. glabrata* sensu stricto. There were no significant
151 differences in the species distribution by gender or age (for a *p* value of 0.05).

152 **Species susceptibility profiles** Table 2 summarizes susceptibility testing results;
153 endpoints shown were obtained after 24h of incubation and they differ no more than a
154 single dilution from those obtained after 48h. Global antifungal resistance rates were
155 calculated (considering the five main species, except for FLC, where *C. krusei* were
156 excluded on the basis of intrinsic resistance; for the echinocandins the four isolates of
157 *C. guilliermondii* were taken into account; for POS, epidemiological cutoff values were
158 used). Among the group of azoles, the highest resistance rate was observed for POS
159 (18.5%), followed by VRC (15.6%) and FLC (13.1%). Concerning the echinocandins, CAS
160 was the drug with the most resistances (17.2% of isolates), surpassing AND (14.0%)

161 and MCF (11.6%). Resistance to 5FC was rare. In the matter of simultaneous resistance
162 to antifungals of different classes (excluding strains with intrinsic resistances such as *C.*
163 *krusei* for FLC and *C. neoformans* to echinocandins), we detected one isolate of *T.*
164 *mucoides* (resistant to FLC, AND, CAS and MCF), one of *C. krusei* (resistant to AMB and
165 CAS) and one of *C. parapsilosis* (resistant to FLC and CAS). Resistance to drugs within
166 the same class was more frequent, with 8 strains of *C. albicans* and 1 of *C. tropicalis*
167 displaying resistance to the three azoles and 6 strains of *C. parapsilosis* resistant to the
168 three echinocandins (MIC \geq 8mg/L). Regarding the *in vitro* activity of AMB, with the
169 exception of three isolates (one each of *C. glabrata*, *C. krusei* and *C. kefyr*) which
170 corresponded to a high MIC value (2 mg/L), all the other strains were susceptible with
171 very low MIC values, ranging from 0.03 to 0.125 mg/L. Susceptibility testing results
172 revealed *C. parapsilosis* as the most resistant species to echinocandins, with only 20%
173 of the isolates susceptible to the 3 antifungals (resistance rate of 31% to MCF, 53% to
174 AND and 51% to CAS). On the other hand, *C. albicans* displayed high susceptibility to
175 these drugs but considerable resistance to azoles (18.6% to FLC, 18.6% to VRC and
176 16.5% to POS). Almost one third of *C. glabrata* exhibited a high MIC of VRC, with lower
177 values to other azoles (6.25% for POS and FLC) and echinocandins (18.75% for MCF,
178 9.4% to CAS and 0% to AND). All *C. tropicalis* isolates were susceptible to
179 echinocandins, but there were resistances to azole antifungals (60% to POS, 33% to
180 VRC and 20% to FLC). *C. krusei* was susceptible to the azoles (apart from the expected
181 resistance to FLC), AND and MCF. High MIC values to CAS were found in 5 isolates
182 (41.6%). All the participating hospitals had Intensive Care Units (ICU) and Pediatric
183 Departments and although very distinct geographic areas were covered by these

184 hospitals, no significant differences in species distribution and the antifungal
185 susceptibility profile were found (for $p=0.05$).

186 DISCUSSION

187 Our study reports an incidence of fungemia of 0.88/1000 admissions (ranging
188 from 0.15 to 2.4), figures comparable to recent data from other European countries
189 (12, 13) albeit somewhat lower than in Brazil (14). A previous study in Portugal, based
190 on a single university hospital, uncovered a similar, though slightly higher, incidence of
191 2.7/1000 admissions (15). Healthcare-associated fungemia remains the vast majority,
192 representing more than 80% in most works (16, 17). Thus, it is not unsurprising that
193 most of the isolates were recovered from ICU, surgery and internal medicine
194 departments, given that most patients admitted to these wards are in critical health
195 conditions and are submitted to invasive procedures, aggressive antibiotic and
196 immunosuppressive drug regimens and placement of indwelling devices (central and
197 peripheral venous catheters, urinary catheters and invasive ventilation procedures), all
198 previously associated with increased rates of fungal infections (18-21). We report a
199 crude 30-day mortality rate of 25%, with more than half (53%) of *C. glabrata*
200 fungemias being lethal. Costa-de-Oliveira et al. reported, five years ago, even higher
201 values, with a 78% mortality rate for *C. glabrata* fungemia and 46%, 30% and 53% for
202 *C. albicans*, *C. parapsilosis* and *C. tropicalis*, respectively (15). The reasons behind this
203 decrease can only be speculated. In other European studies crude mortality rates due
204 to fungemia ranged from around 30% (12, 16) to 40%(17, 22), but *C. glabrata* did not
205 carry a heavier death burden. *C. albicans* appears as the leading causative agent of
206 fungemia in our study (40%) and in other European studies, accounting for an even

207 higher proportion of cases (12, 16, 22-24). However, the second position is not
208 occupied by *C. parapsilosis* in most European countries; instead, *C. glabrata* appears
209 repeatedly as the most common non-*albicans* species isolated from blood cultures (2),
210 reaching almost a third of all *Candida* strains in some studies (17, 23). In fact, some
211 authors place *C. parapsilosis* in the fourth (16) or even fifth position (23), behind *C.*
212 *tropicalis* and *C. krusei*. The causes of this difference in incidences are unknown to us.
213 Curiously, our data parallels those of Spanish and Brazilian works, where *C. parapsilosis*
214 is responsible for 27 and 37% of cases of fungemia (13, 25). Taking into account the
215 recent separation of the cryptic species in the *C. parapsilosis* and *C. glabrata*
216 complexes into species in their own right, we strived to identify these in our studies. *C.*
217 *orthopsilosis* and *C. metapsilosis* represented 7.3 and 3.6%, respectively, of the *C.*
218 *parapsilosis* sensu lato isolates, while *C. nivariensis* and *C. bracarensis* were not
219 identified, coinciding with results in other Mediterranean countries (13, 26, 27) and
220 Brazil (25). In our work we applied the new CLSI M27-S4 breakpoints for antifungal
221 susceptibilities (9), which may have altered the final resistance values for the five main
222 species. Comparison with other studies is hindered by the fact that most prior works
223 do not use these breakpoints and a large part of those that do does not present the
224 susceptibility results as MIC distribution in the range of tested antifungal
225 concentrations. Our work does show relatively high resistance rates to azoles and
226 echinocandins, compared to other countries (28). However, it is important to highlight
227 the small size of our sample and point out that more isolates (either through greater
228 participation or by extending the period of the study) would strengthen our results.
229 Mortality due to fungemia remains at unacceptably high values. Studies like the one
230 we present (the first multicenter study carried out in Portugal) are of utmost

231 importance, for they provide invaluable data on the species distribution and antifungal
232 susceptibility, and can guide clinicians in the treatment of these infections.

233

234 ACKNOWLEDGEMENTS

235 We would like to thank all the participants that have contributed with isolates and
236 clinical data: J. Melo Cristino (Centro Hospitalar de Lisboa Norte); V. Lopes, J. Teixeira
237 and H. Ramos (Centro Hospitalar do Porto); C. Toscano and T. Marques (Centro
238 Hospitalar de Lisboa Ocidental); Z. Videira, A. Almeida, E. Tiza and M. Silveira (Instituto
239 Português de Oncologia de Lisboa); G. Gonçalves and A. Estrada (Hospital de São
240 Marcos, Braga); A.C. Lameiras and M.A. Guimarães (Instituto Português de Oncologia
241 do Porto); H. Oliveira and M. H. Pereira (Centro Hospitalar de Coimbra); E. Ramalheira
242 and S. Ferreira (Hospital Infante Dom Pedro, Aveiro).

243 Part of the results was previously presented at the 23rd European Congress of Clinical
244 Microbiology and Infectious Diseases held in Berlin, Germany, April 2013.

245

246 FUNDING

247 This work was supported by Pfizer Inc. Grant WS759839 and also had the collaboration
248 and support of “Grupo de Estudos de Micologia Médica” (GEMM) and PTDC/DTP –
249 EPI/1660/2012 “Surveillance of the emergent *Candida parapsilosis* antifungal
250 resistance”.

251

252 TRANSPARENCY DECLARATIONS

253 No conflicts to declare.

254

255 REFERENCES

- 256 1. **Garrouste-Orgeas M, Timsit JF, Tafflet M, Misset B, Zahar JR, Soufir L, Lazard**
257 **T, Jamali S, Mourvillier B, Cohen Y, De Lassece A, Azoulay E, Cheval C,**
258 **Descorps-Declere A, Adrie C, Costa de Beauregard MA, Carlet J, Group OS.**
259 2006. Excess risk of death from intensive care unit-acquired nosocomial
260 bloodstream infections: a reappraisal. Clinical infectious diseases : an official
261 publication of the Infectious Diseases Society of America **42:1118-1126.**
- 262 2. **Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, Rodloff A, Fu**
263 **W, Ling TA, Global Antifungal Surveillance G.** 2010. Results from the ARTEMIS
264 DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of
265 susceptibilities of Candida Species to fluconazole and voriconazole as
266 determined by CLSI standardized disk diffusion. Journal of clinical microbiology
267 **48:1366-1377.**
- 268 3. **Fortun J, Martin-Davila P, Gomez-Garcia de la Pedrosa E, Pintado V, Cobo J,**
269 **Fresco G, Meije Y, Ros L, Alvarez ME, Luengo J, Agundez M, Belso A, Sanchez-**
270 **Sousa A, Loza E, Moreno S.** 2012. Emerging trends in candidemia: a higher
271 incidence but a similar outcome. The Journal of infection **65:64-70.**
- 272 4. **Liu CY, Liao CH, Chen YC, Chang SC.** 2010. Changing epidemiology of
273 nosocomial bloodstream infections in 11 teaching hospitals in Taiwan between

- 274 1993 and 2006. Journal of microbiology, immunology, and infection = Wei mian
275 yu gan ran za zhi **43**:416-429.
- 276 5. **Playford EG, Nimmo GR, Tilse M, Sorrell TC.** 2010. Increasing incidence of
277 candidaemia: long-term epidemiological trends, Queensland, Australia, 1999-
278 2008. The Journal of hospital infection **76**:46-51.
- 279 6. **Romeo O, Scordino F, Pernice I, Lo Passo C, Criseo G.** 2009. A multiplex PCR
280 protocol for rapid identification of *Candida glabrata* and its phylogenetically
281 related species *Candida nivariensis* and *Candida bracarensis*. Journal of
282 microbiological methods **79**:117-120.
- 283 7. **Tavanti A, Davidson AD, Gow NA, Maiden MC, Odds FC.** 2005. *Candida*
284 orthopsilosis and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis*
285 groups II and III. Journal of clinical microbiology **43**:284-292.
- 286 8. **Rex JH, Alexander BD, Andes D, Arthington-Skaggs B, Brown SD, Chaturvedi V,**
287 **Ghannoum M, Espinel-Ingroff A, Knapp CC, Ostrosky-Zeichner L, Pfaller MA,**
288 **Sheehan DJ, Walsh TJ.** 2008. Reference Method for Broth Dilution Antifungal
289 Susceptibility Testing of Yeasts: Approved standard - Third Edition. CLSI
290 Document M27-A3.
- 291 9. **CLSI.** 2012. Reference Method for Broth Dilution Antifungal Susceptibility
292 Testing of Yeasts: Fourth Informational Supplement. CLSI Document M27-S4.
- 293 10. **Pfaller MA, Boyken L, Hollis RJ, Kroeger J, Messer SA, Tendolkar S, Diekema**
294 **DJ.** 2011. Wild-type MIC distributions and epidemiological cutoff values for
295 posaconazole and voriconazole and *Candida* spp. as determined by 24-hour
296 CLSI broth microdilution. Journal of clinical microbiology **49**:630-637.

- 297 11. **Pfaller MA, Espinel-Ingroff A, Canton E, Castanheira M, Cuenca-Estrella M,**
298 **Diekema DJ, Fothergill A, Fuller J, Ghannoum M, Jones RN, Lockhart SR,**
299 **Martin-Mazuelos E, Melhem MS, Ostrosky-Zeichner L, Pappas P, Pelaez T,**
300 **Peman J, Rex J, Szeszs MW.** 2012. Wild-type MIC distributions and
301 epidemiological cutoff values for amphotericin B, flucytosine, and itraconazole
302 and *Candida* spp. as determined by CLSI broth microdilution. *Journal of clinical*
303 *microbiology* **50**:2040-2046.
- 304 12. **Tortorano AM, Prigitano A, Lazzarini C, Passera M, Deiana ML, Cavinato S, De**
305 **Luca C, Grancini A, Lo Cascio G, Ossi C, Sala E, Montagna MT.** 2013. A 1-year
306 prospective survey of candidemia in Italy and changing epidemiology over one
307 decade. *Infection* **41**:655-662.
- 308 13. **Peman J, Canton E, Quindos G, Eraso E, Alcoba J, Guinea J, Merino P, Ruiz-**
309 **Perez-de-Pipaon MT, Perez-del-Molino L, Linares-Sicilia MJ, Marco F, Garcia J,**
310 **Rosello EM, Gomez Gd-I-PE, Borrell N, Porras A, Yague G, Group FS.** 2012.
311 Epidemiology, species distribution and in vitro antifungal susceptibility of
312 fungemia in a Spanish multicentre prospective survey. *The Journal of*
313 *antimicrobial chemotherapy* **67**:1181-1187.
- 314 14. **Colombo AL, Nucci M, Park BJ, Nouer SA, Arthington-Skaggs B, da Matta DA,**
315 **Warnock D, Morgan J, Brazilian Network Candidemia S.** 2006. Epidemiology of
316 candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven
317 medical centers. *Journal of clinical microbiology* **44**:2816-2823.
- 318 15. **Costa-de-Oliveira S, Pina-Vaz C, Mendonca D, Goncalves Rodrigues A.** 2008. A
319 first Portuguese epidemiological survey of fungemia in a university hospital.

- 320 European journal of clinical microbiology & infectious diseases : official
321 publication of the European Society of Clinical Microbiology **27**:365-374.
- 322 16. **Asmundsdottir LR, Erlendsdottir H, Gottfredsson M.** 2013. Nationwide study
323 of candidemia, antifungal use, and antifungal drug resistance in Iceland, 2000
324 to 2011. Journal of clinical microbiology **51**:841-848.
- 325 17. **Das I, Nightingale P, Patel M, Jumaa P.** 2011. Epidemiology, clinical
326 characteristics, and outcome of candidemia: experience in a tertiary referral
327 center in the UK. International journal of infectious diseases : IJID : official
328 publication of the International Society for Infectious Diseases **15**:e759-763.
- 329 18. **Wu Z, Liu Y, Feng X, Liu Y, Wang S, Zhu X, Chen Q, Pan S.** 2014. Candidemia:
330 incidence rates, type of species, and risk factors at a tertiary care academic
331 hospital in China. International journal of infectious diseases : IJID : official
332 publication of the International Society for Infectious Diseases.
- 333 19. **Wang H, Wu DW, Han H, Yue JF, Zhang F, Shan TC, Guo HP, Yin M.** 2014.
334 Antibiotics exposure, risk factors, and outcomes with *Candida albicans* and non-
335 *Candida albicans* candidemia. Results from a multi-center study. Saudi medical
336 journal **35**:153-158.
- 337 20. **Quindos G.** 2014. Epidemiology of candidaemia and invasive candidiasis. A
338 changing face. Revista iberoamericana de micologia **31**:42-48.
- 339 21. **Adukauskiene D, Kinderyte A, Dambrauskiene A, Vitkauskiene A.** 2009.
340 [Candidemia in an intensive care unit]. Medicina **45**:351-356.
- 341 22. **Chalmers C, Gaur S, Chew J, Wright T, Kumar A, Mathur S, Wan WY, Gould IM,**
342 **Leanord A, Bal AM.** 2011. Epidemiology and management of candidaemia--a

- 343 retrospective, multicentre study in five hospitals in the UK. *Mycoses* **54**:e795-
344 800.
- 345 23. **Arendrup MC, Dzajic E, Jensen RH, Johansen HK, Kjaeldgaard P, Knudsen JD,**
346 **Kristensen L, Leitz C, Lemming LE, Nielsen L, Olesen B, Rosenvinge FS, Roder**
347 **BL, Schonheyder HC.** 2013. Epidemiological changes with potential implication
348 for antifungal prescription recommendations for fungemia: data from a
349 nationwide fungemia surveillance programme. *Clinical microbiology and*
350 *infection : the official publication of the European Society of Clinical*
351 *Microbiology and Infectious Diseases* **19**:E343-353.
- 352 24. **Ericsson J, Chryssanthou E, Klingspor L, Johansson AG, Ljungman P, Svensson**
353 **E, Sjolín J.** 2013. Candidaemia in Sweden: a nationwide prospective
354 observational survey. *Clinical microbiology and infection : the official*
355 *publication of the European Society of Clinical Microbiology and Infectious*
356 *Diseases* **19**:E218-221.
- 357 25. **Bonfietti LX, Szeszs MW, Chang MR, Martins MA, Pukinskas SR, Nunes MO,**
358 **Pereira GH, Paniago AM, Purisco SU, Melhem MS.** 2012. Ten-year study of
359 species distribution and antifungal susceptibilities of *Candida* bloodstream
360 isolates at a Brazilian tertiary hospital. *Mycopathologia* **174**:389-396.
- 361 26. **Tosun I, Akyuz Z, Guler NC, Gulmez D, Bayramoglu G, Kaklikkaya N, Arikan-**
362 **Akdagli S, Aydin F.** 2013. Distribution, virulence attributes and antifungal
363 susceptibility patterns of *Candida parapsilosis* complex strains isolated from
364 clinical samples. *Med Mycol* **51**:483-492.
- 365 27. **Esposto MC, Prigitano A, Romeo O, Criseo G, Trovato L, Tullio V, Fadda ME,**
366 **Tortorano AM, Group FW.** 2013. Looking for *Candida nivariensis* and *C.*

367 bracarensis among a large Italian collection of *C. glabrata* isolates: results of the
368 FIMUA working group. *Mycoses* **56**:394-396.

369 28. **Orasch C, Marchetti O, Garbino J, Schrenzel J, Zimmerli S, Muhlethaler K,**
370 **Rossi M, Pfyffer G, Rued C, Fehr J, Zbinden R, Calandra T, Bille J, The Fungal**
371 **Infection Network of S.** 2013. *Candida* Species Distribution and Antifungal
372 Susceptibility Testing According to EUCAST and New vs. Old CLSI Clinical
373 Breakpoints: a Six-Year Prospective Candidemia Survey from the Fungal
374 Infection Network of Switzerland (FUNGINOS). *Clinical microbiology and*
375 *infection* : the official publication of the European Society of Clinical
376 Microbiology and Infectious Diseases.

377

378

379

Table 1 - Distribution of the isolated yeast strains

	No. of isolates (%)						
	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>C. glabrata</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	Other species	Overall
Gender							
Male	59 (61)	26 (47)	21 (66)	11 (73)	4 (33)	16 (55)	137 (57)
Female	38 (39)	29 (53)	11 (34)	4 (27)	8 (67)	13 (45)	103 (43)
Total	97 (40)	55 (23)	32 (13)	15 (6)	12 (5)	29 (12)	240 (100)
Age group (years)							
<20	8 (8)	13(23)	1(3)	-	-	12 (42)	34 (14)
20-40	10 (10)	4(7)	3(9)	2(13)	2(17)	6 (21)	27 (11)
41-60	35 (35)	13(24)	9(29)	7(47)	6(50)	7 (24)	77 (32)
61-70	15 (16)	7(13)	3(9)	3(20)	1(8)	2 (7)	31 (13)
>70	29 (30)	18(32)	16(50)	3(20)	3(25)	2 (7)	71 (30)
Hospital Department							
ICU	42(43)	21(38)	12 (38)	4(27)	5(42)	10 (35)	94(39)
Surgery	22 (23)	18(33)	8(25)	8(53)	3(25)	11 (38)	70(30)
Medicine	28 (29)	13(24)	11(34)	3(20)	4(33)	6 (21)	65(27)
Pediatrics	5 (5)	3 (6)	1(3)	-	-	2 (7)	11 (5)
Outcome							
Death 30 days	23 (24)	12 (22)	17 (53)	3 (20)	-	4 (14)	59 (25)

380
381

382 Table 2 – Distribution of MIC values according to the species

Species (no.isolates)	Drug	R breakpoint or ECV	No. of isolates with MIC (mg/L)													No (%) of Resistance	
			0,015	0,03	0,06	0,125	0,25	0,5	1	2	4	8	16	32	64		128
<i>C. albicans</i> (97)	AND	>0.5	91	2	3						1		-	-	-	-	1 (1)
	CAS	>0.5		86	1	1	5	3	1				-	-	-	-	1 (1)
	MCF	>0.5	82	1	3	2	4	3		1		1	-	-	-	-	2 (2.1)
	FLC	>4	-	-	-	8	31	24	15	1		17			1		18 (18.6)
	VOR	>0.5	-	58	7	11	1	2		2	12	4		-	-	-	18 (18.6)
	POS	>0.06*	-	81		1						4	11	-	-	-	16 (16.5)
	AMB	>1	11	21	54	1	2	4	4					-	-	-	0
	5FC	>0.5*	-	-	-	61	18	10	5	2						1	-
<i>C. parapsilosis</i> (55)	AND	>4				1	1	4	1	6	13	19	10	-	-	-	29 (52.7)
	CAS	>4		1	2	1	1	3	12		7	20	8	-	-	-	28 (50.9)
	MCF	>4		4		8	2	4	9	3	8	6	8	3	-	-	17 (30.9)
	FLC	>4	-	-	-		16	27	4	2	3	1	2				3 (5.5)
	VOR	>0.5		17		23	8	7						-	-	-	0
	POS	>0.25*			31	12		9	2	1				-	-	-	12 (21.8)
	AMB	>1		42	8	4	1							-	-	-	0
	5FC	>0.5*	-	-	-	49	3	2	1								1 (1.8)
<i>C. glabrata</i> (32)	AND	>0.25		19	8	3	2						-	-	-	-	0
	CAS	>0.25		13	9	3	4	2			1		-	-	-	-	3 (9.4)
	MCF	>0.12		21		5		2	3		1		-	-	-	-	6 (18.75)
	FLC	>32	-	-	-			2	1	8	7	12			2		2 (6.25)
	VOR	>0.5*			8	3	9	2	6		3	1		-	-	-	10 (31.25)
	POS	>2*			4	5	5	3	9	4		2		-	-	-	2 (6.25)
	AMB	>1		4	9	7	6	3	2	1							1 (3.1)
	5FC	>0.5*	-	-	-	22	2	5	3								3 (9.4)
<i>C. tropicalis</i> (15)	AND	>0.5		7	3	4	1						-	-	-	-	0
	CAS	>0.5		6	3	2	4						-	-	-	-	0
	MCF	>0.5		3		5	3	4					-	-	-	-	0
	FLC	>4	-	-	-	5	3	3		1	2				1		3 (20)
	VOR	>0.5		1	3	1	5		3		2			-	-	-	5 (33.3)
	POS	>0.12*		2	1	3	4	1	1		2	1		-	-	-	9 (60)

	AMB	>1		12	3											0
	5FC	>0.5*				6	4	3			1	1				2 (13.3)
<i>C. krusei</i> (12)	AND	>0.5		8		2	2						-	-	-	0
	CAS	>0.5				3	2	2	3	1	1		-	-	-	5 (41.6)
	MCF	>0.5				3	6	3					-	-	-	0
	FLC	>64*											2	7	3	-
	VOR	>1		6	5	1								-	-	0
	POS	>0.5*		3	1	1	7							-	-	0
	AMB	>1		9	1		1			1						1 (8.3)
	5FC	>32*									2	3	6	1		0
Other spp. (29)	AND	>2	5	4	1	12	1	1	3	1	1					1
	CAS	>2	6	1	4	3	1	10	1	2	1					1
	MCF	>2	4	2	5	2	3	2	4	4	2	1				3
	FLC	>64		5	1	4	3	6	5		1		3		1	1
	VOR	>4		15	2	3	6	1	2							0
	POS	>4		17	5	3	2	1			1			-		0
	AMB	>1		4	2	7	7	7	1	1						1
	5FC	>32			5	8	5	6	2	2				1		1
Overall (240)	AND	NA	96	40	15	22	7	5	4	7	15	19	10			
	CAS	NA	6	107	19	13	17	20	17	3	10	20	8			
	MCF	NA	86	31	8	25	18	18	16	8	11	8	8	3		
	FLC	NA		5	1	17	53	62	25	11	12	32	7	7	8	
	VOR	NA		97	25	42	29	12	11	2	17	5				
	POS	NA		103	42	25	18	14	12	5	3	7	11			
	AMB	NA	11	92	77	19	17	14	7	3						
	5FC	NA			5	146	32	26	11	4	3	4	6	2	1	

383

*ECV value

NA = not applicable; Resistant strains are **boldface**

AGRADECIMENTOS

Os meus sinceros agradecimentos,

à minha orientadora, a Professora Doutora Cidália Pina Vaz, pelo seu apoio, motivação e conhecimentos, indispensáveis à realização deste trabalho.

aos outros autores deste trabalho, em especial à Dra. Isabel Faria Ramos, pelo seu companheirismo, dedicação e inesgotável paciência.

à Professora Doutora Emília Cantón, pelo seu incansável trabalho de revisão e correcção, que tanto melhorou este trabalho.

ao Departamento de Microbiologia da Faculdade de Medicina da Universidade do Porto.

Normas da Revista

Journal of Clinical Microbiology

2014 INSTRUCTIONS TO AUTHORS

SCOPE

The *Journal of Clinical Microbiology* (JCM) is devoted to the dissemination of new knowledge concerning the laboratory diagnosis of human and animal infections. In addition, JCM is an appropriate forum for the publication of information related to the role of the laboratory in both the management of infectious diseases and the elucidation of the epidemiology of infections. Manuscripts which present the results of original scientific investigations are encouraged. The three principal attributes that we require of papers published in JCM are timeliness, relevance to the practice of clinical microbiology, and quality science. Manuscripts that present information that is largely only of relevance to a restricted geographic area are discouraged.

JCM welcomes submission of manuscripts that present the results of investigations pertaining to new technologies in clinical microbiology when they address new applications, substantially extend our understanding of the role of the technology in the clinical microbiology laboratory, or provide clinical or laboratory outcome data.

JCM will consider manuscripts which describe truly novel molecular methods for use in the diagnosis or elucidation of infection. However, we discourage submission of manuscripts predicated on the application of previously well-described methods (e.g., RT-PCR, RAPD analysis, PFGE, and real-time PCR, etc.), even though the application may be new. Papers employing established molecular methods will be considered only when the application is examined in comparison to some other existing diagnostic method(s). Such comparative studies should include information regarding assay sensitivity, specificity, and diagnostic accuracy.

Studies with matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) as a primary focus must offer something that is truly novel in order to merit consideration for publication in JCM, e.g., application of MALDI-TOF MS to previously unstudied organisms or organism groups, applications that extend beyond organism identification, clinical outcome studies, comparisons of different MALDI-TOF instruments, broad comparisons with conventional methods, or descriptions of modifications of existing MALDI-TOF MS methods. JCM will not consider manuscripts that describe routine applications of MALDI-TOF MS.

Papers submitted to JCM with whole-genome sequencing or the microbiome as a focus will be considered only when the studies performed are scientifically sound, the observations timely and novel, and the information presented of relevance to the practice of clinical microbiology. Similarly, papers that convey the results of meta-analysis studies will be considered when the data presented are extensive and when the observations made in the study are broadly applicable to the practice of clinical microbiology.

Case Reports will be considered if they are novel, add to existing knowledge, and are consistent with the primary objectives of the *Journal* as outlined above. (See “Case Reports” in the [Organization and Format](#) section and the “Case Report”

paragraph under “Full-Length Papers” for descriptions of the two different types of Case Reports published.)

To ensure the completeness of investigations in which the performance of various diagnostic assays or methods is presented, authors are encouraged to refer to the Standards for the Reporting of Diagnostic Accuracy (STARD) for guidance. The entire set of guidelines, including checklists, may be found at <http://stard-statement.org/>.

ASM publishes a number of different journals covering various aspects of the field of microbiology. Each journal has a prescribed scope which must be considered in determining the most appropriate journal for each manuscript.

(i) With respect to antimicrobial agents, JCM will consider clinically relevant manuscripts (a) that pertain to *in vitro* susceptibility test methods; (b) that are concerned with quality control procedures related to antimicrobial susceptibility tests; (c) that deal with investigations of test methods aimed at measuring levels of antimicrobial agents in clinical specimens; or (d) that describe the use of antimicrobial agents as tools in the isolation, identification, or epidemiologic assessment of microorganisms associated with disease. Manuscripts pertaining to other aspects of antimicrobial agents, such as their basic mechanisms of action, the elucidation of resistance determinants, pharmacokinetics and pharmacodynamics, and the development of new agents, will be considered for publication in *Antimicrobial Agents and Chemotherapy*.

(ii) Manuscripts that present the results of investigations with a primary focus on the basic mechanisms of pathogenesis of microorganisms or the pathophysiology of infections should be directed to *Infection and Immunity* (for bacteria, parasites, and fungi) or the *Journal of Virology* (for viruses).

(iii) Reports of clinical microbiology investigations or studies of the hospital population and the environment as they relate to nosocomial infections should be submitted to JCM. Manuscripts dealing with ecology or environmental studies or with the application of microorganisms to agricultural or industrial processes are more appropriate for *Applied and Environmental Microbiology*.

(iv) Papers involving immunologic assays for use in the diagnosis or elucidation of infection, vaccines, or the assessment and laboratory diagnosis of immunologic diseases (e.g., autoimmune diseases and primary immunodeficiencies) should be submitted to *Clinical and Vaccine Immunology* (formerly *Clinical and Diagnostic Laboratory Immunology*).

Questions about these guidelines may be directed to the editor in chief of the journal being considered.

If transfer to another ASM journal is recommended by an editor, the corresponding author will be contacted.

Note that a manuscript rejected by one ASM journal on scientific grounds or on the basis of its general suitability for publication is considered rejected by all other ASM journals.

EDITORIAL POLICY

Use of Microbiological Information

The Council Policy Committee (CPC) of the American Society for Microbiology affirms the long-standing position of the Society that microbiologists will work for the proper and beneficent application of science and will call to the attention of the public or the appropriate authorities misuses of microbiology or of information derived from microbiology. ASM members are obligated to discourage any use of microbiology contrary to the welfare of humankind, including the use of microbes as biological weapons. Bioterrorism violates the fundamental principles expressed in the Code of Ethics of the Society and is abhorrent to ASM and its members.

ASM recognizes that there are valid concerns regarding the publication of information in scientific journals that could be put to inappropriate use as described in the CPC resolution mentioned above. Members of the ASM Journals Board will evaluate the rare manuscript that might raise such issues during the review process. However, as indicated elsewhere in these Instructions, research articles must contain sufficient detail, and material/information must be made available, to permit the work to be repeated by others. Supply of materials should be in accordance with laws and regulations governing the shipment, transfer, possession, and use of biological materials and must be for legitimate, bona fide research needs. Links to, and information regarding, these laws and regulations can be found at <http://www.asm.org/> under the Public Policy tab. We ask that authors pay particular attention to the NSAR Select Agents and Toxins list on the CDC website <http://www.selectagents.gov/index.html> and the NSABB criteria for identifying dual use research of concern in the report “Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information” on the Office of Biotechnology Activities website http://oba.od.nih.gov/biosecurity/pdf/Framework%20for%20transmittal%200807_Sept07.pdf (p. 17–22).

Ethical Guidelines

ASM requirements for submitted manuscripts are consistent with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, as last updated by the International Committee of Medical Journal Editors in April 2010 (<http://www.icmje.org/>).

Authors are expected to adhere to the highest ethical standards. The following sections of these Instructions include detailed information about ASM’s ethical standards. Failure to comply with the policies described in these Instructions may result in a letter of reprimand, a suspension of publishing privileges in ASM journals, and/or notification of the authors’ institutions. Authors employed by companies whose policies do not permit them to comply with ASM policies may be sanctioned as individuals and/or ASM may refuse to consider man-

uscripts having authors from such companies. The ASM Journals Board wishes to clarify the following in particular.

Plagiarism. Misappropriating another person’s intellectual property constitutes plagiarism. This includes copying sentences or paragraphs verbatim (or almost verbatim) from someone else’s work, even if the original work is cited in the references. The NIH ORI publication “Avoiding Plagiarism, Self-Plagiarism, and Other Questionable Writing Practices: a Guide to Ethical Writing” (<http://ori.dhhs.gov/avoiding-plagiarism-self-plagiarism-and-other-questionable-writing-practices-guide-ethical-writing>) can help authors identify questionable writing practices.

Plagiarism is not limited to the text; it can involve any part of the manuscript, including figures and tables, in which material is copied from another publication without permission and attribution. An author may not reuse his or her own previously published work without attribution; this is considered self-plagiarism.

Primary publication. Manuscripts submitted to the journal must represent reports of original research, and the original data must be available for review by the editor if necessary.

By submission of a manuscript to the journal, **the authors guarantee that they have the authority to publish the work and that the manuscript, or one with substantially the same content, was not published previously, is not being considered or published elsewhere, and was not rejected on scientific grounds by another ASM journal.** It is incumbent upon the author to acknowledge any prior publication, including his/her own articles, of the data contained in a manuscript submitted to an ASM journal. A copy of the relevant work should be submitted with the paper as supplemental material for information only. Whether the material constitutes the substance of a paper and therefore renders the manuscript unacceptable for publication is an editorial decision.

In brief, a paper is not acceptable for submission to an ASM journal if it, or its substance, has been made publicly available in:

- A serial, periodical, or book
- A conference report or symposium proceedings
- A technical bulletin or company white paper
- A public website
- Any other retrievable source

The following do not preclude submission to, or publication by, an ASM journal, as long as the posted data do not constitute the substance of a submission:

- Posting of a method/protocol on a public website
- Posting of a limited amount of original data on a personal/university/corporate website or websites of small collaborative groups working on a problem
- Deposit of unpublished sequence data in a public database
- Preliminary disclosures of research findings as meeting posters, webcast as meeting presentations, or published in abstract form as adjuncts to a meeting, e.g., part of a program

- Posting of theses and dissertations on a personal/university-hosted website

Availability of materials. By publishing in the journal, the authors agree that, subject to requirements or limitations imposed by laws or governmental regulations of the United States, any DNAs, viruses, microbial strains, mutant animal strains, cell lines, antibodies, and similar materials described in the article are available from a national collection or will be made available in a timely fashion, at reasonable cost, and in limited quantities to members of the scientific community for noncommercial purposes. The authors guarantee that they have the authority to comply with this policy either directly or by means of material transfer agreements through the owner.

Similarly, the authors agree to make available computer programs, originating in the authors' laboratory, that are the only means of confirming the conclusions reported in the article but that are not available commercially. The program(s) and suitable documentation regarding its (their) use may be provided by any of the following means: (i) as a program transmitted via the Internet, (ii) as an Internet server-based tool, or (iii) as a compiled or assembled form on a suitable medium. It is expected that the material will be provided in a timely fashion and at reasonable cost to members of the scientific community for noncommercial purposes. The authors guarantee that they have the authority to comply with this policy either directly or by means of material transfer agreements through the owner.

Permissions. The corresponding author is responsible for obtaining permission from both the original author and the original publisher (i.e., the copyright owner) to reproduce or modify figures (including maps) and tables and to reproduce text (in whole or in part) from previous publications.

Permission(s) must be obtained no later than the modification stage. The original signed permission(s) must be identified as to the relevant item in the ASM manuscript (e.g., "permissions for Fig. 1 in JCM00123-14") and submitted to the ASM production editor on request. In addition, a statement indicating that the material is being reprinted with permission must be included in the relevant figure legend or table footnote of the manuscript. Reprinted text must be enclosed in quotation marks, and the permission statement must be included as running text or indicated parenthetically.

It is expected that the authors will provide written assurance that permission to cite unpublished data or personal communications has been granted. For supplemental material intended for posting by ASM (see "[Supplemental Material](#)"), if the authors of the JCM manuscript are not also the owners of the supplemental material, the corresponding author must send to ASM signed permission from the copyright owner that allows posting of the material, as a supplement to the article, by ASM. The corresponding author is also responsible for incorporating in the supplemental material any copyright notices required by the owner.

Authorship. All authors of a manuscript must have agreed to its submission and are responsible for its content (initial submission and any subsequent versions), including appropri-

ate citations and acknowledgments, and must also have agreed that the corresponding author has the authority to act on their behalf in all matters pertaining to publication of the manuscript. The corresponding author is responsible for obtaining such agreements and for informing the coauthors of the manuscript's status throughout the submission, review, and publication process. Submitting a paper before all coauthors have read and approved it is considered an ethical violation, as is failure to credit someone who qualifies as a coauthor; however, ASM does not itself investigate or attempt to resolve authorship disputes.

An author is one who made a substantial contribution to the overall design and execution of the experiments; therefore, **ASM considers all authors responsible for the entire paper.** Individuals who provided assistance, e.g., supplied strains or reagents or critiqued the paper, need not be listed as authors but may be recognized in the Acknowledgments section. ASM does not permit "ghost authorship," i.e., individuals who contribute to the research, data analysis, and/or writing of an article but who do not satisfy the requirements for authorship. Examples of ghost authors include medical writers and employees of pharmaceutical or device companies who have not made a substantial contribution to the overall design and execution of the experiments.

A study group, surveillance team, working group, consortium, or the like (e.g., the Active Bacterial Core Surveillance Team) may be listed as a coauthor in the byline if its contributing members satisfy the requirements for authorship and accountability as described in these Instructions. The names (and institutional affiliations if desired) of the contributing members only may be given as a separate paragraph in the Acknowledgments section.

If the contributing members of the group associated with the work do not fulfill the criteria of substantial contribution to and responsibility for the paper, the group may not be listed in the author byline. Instead, it and the names of its contributing members may be listed in the Acknowledgments section.

All authors must agree to the order in which their names are listed in the byline. Statements regarding equal contributions by two or more authors (e.g., "C.J. and Y.S. contributed equally to . . .") are permitted as footnotes and must be agreed to by all of the authors. Other statements of attribution may be included in the Acknowledgments section.

A change in authorship (order of listing, addition or deletion of a name, or corresponding author designation) after submission of the manuscript will be implemented only after receipt of signed statements of agreement from all parties involved.

Disputes about authorship may delay or prevent review and/or publication of the manuscript. Should the individuals involved be unable to reach an accord, review and/or publication of the manuscript can proceed only after the matter is investigated and resolved by the authors' institution(s) and an official report of such and signed statements of agreement are provided to ASM.

Conflict of interest. All authors are expected to disclose, in the manuscript submittal letter, any commercial affiliations as well as consultancies, stock or equity interests, and patent-licensing arrangements that could be considered to pose a con-

flict of interest regarding the submitted manuscript. (Inclusion of a company name in the author address lines of the manuscript does not constitute disclosure.) Details of the disclosure to the editor will remain confidential. However, it is the responsibility of authors to provide, in the Acknowledgments section, a general statement disclosing financial or other relationships that are relevant to the study. Examples of potentially conflicting interests that should be disclosed include relationships that might detract from an author's objectivity in presentation of study results and interests whose value would be enhanced by the results presented. All funding sources for the project, institutional and corporate, should be credited in the Acknowledgments section, as described below. In addition, if a manuscript concerns a commercial product, the manufacturer's name must be indicated in the Materials and Methods section or elsewhere in the text, as appropriate, in an obvious manner.

Copyright

To maintain and protect the Society's ownership and rights and to continue to afford scientists the opportunity to publish in high-quality journals, ASM requires the corresponding author to sign a copyright transfer agreement on behalf of all the authors. Unless this agreement is executed (without changes and/or addenda), ASM will not publish the article.

In the copyright transfer agreement signed by an author, ASM grants to that author (and coauthors) the right to republish discrete portions of his/her (their) article in any other publication (print, CD-ROM, and other electronic forms) of which he/she is (they are) the author(s) or editor(s), on the condition that appropriate credit is given to the original ASM publication. This republication right also extends to posting on a host computer to which there is access via the Internet. Except as indicated below, significant portions of the article may not be reprinted/posted without ASM's prior written permission, however, as this would constitute duplicate publication.

Authors may post their own published articles on their personal or university-hosted (but not corporate, government, or similar) websites without ASM's prior written permission provided that appropriate credit is given (i.e., the copyright lines shown at the bottom of the first page).

Works authored solely by U.S. government employees are not subject to copyright protection, so there is no copyright to be transferred. However, the other provisions of the copyright transfer agreement, such as author representations of originality and authority to enter into the agreement, apply to U.S. government employee-authors as well as to other authors.

When funds from the Wellcome Trust or Research Councils UK are used to pay an article open access fee, the article will be published under the [Creative Commons Attribution license \(CC-BY\)](#) in accordance with the funding organization's open access policies. Authors will be required to notify ASM and complete the Author Warranty and Provisional License to Publish at the time of submission.

Copyright for supplemental material (see "[Supplemental Material](#)") remains with the author, but a license permitting the posting by ASM is included in the article copyright transfer agreement. If the author of the article is not also the copyright owner of the supplemental material, the corresponding author must send to ASM signed permission from the owner that

allows posting of the material, as a supplement to the article, by ASM. The corresponding author is also responsible for incorporating into the supplemental material any copyright notices required by the owner.

Funding Agency Repositories

The National Institutes of Health (NIH) requests that its grantee and intramural authors provide copies of their accepted manuscripts to PubMed Central (PMC) for posting in the PMC Public Access Repository. However, JCM authors are automatically in compliance with this policy and need take no action themselves. For the past several years, ASM has deposited in PubMed Central all publications from all ASM journals. Further, ASM policy is that all primary research articles are made available to everyone, free, 6 months after publication through PubMed Central, HighWire, and international PubMed Central-like repositories. By having initiated these policies, ASM is in full compliance with NIH policy. For more information, see <http://publicaccess.nih.gov/>. ASM also allows JCM authors whose work was supported by funding agencies that have public access requirements like those of the NIH (e.g., the Wellcome Trust) to post their accepted manuscripts in publicly accessible electronic repositories maintained by those funding agencies. If a funding agency does not itself maintain such a site, then ASM allows the author to fulfill that requirement by depositing the manuscript (not the typeset article) in an appropriate institutional or subject-based open repository established by a government or noncommercial entity.

Since ASM makes the final, typeset articles from its primary-research journals available free of charge on the ASM Journals and PMC websites 6 months after final publication, ASM requests that when submitting the accepted manuscript to PMC or a similar public access site, the author specify that the **posting release date for the manuscript be no earlier than 6 months after publication of the typeset article by ASM and that a link to the published manuscript on the journal website be provided.**

Use of Human Subjects or Animals in Research

The use of human subjects or animals for research purposes is regulated by the federal government and individual institutions. Authors of manuscripts describing research involving human subjects or animal experimentation must obtain approval from their Institutional Review Board (IRB) or Institutional Animal Care and Use Committee (IACUC), as appropriate, prior to manuscript submission. Authors of manuscripts that describe multisite research must obtain approval from each institution's IRB or IACUC, as appropriate. A statement of IRB or IACUC approval must be included in the Materials and Methods section. Documentation of IRB or IACUC status must be made available upon request.

Patient Identification

To protect the privacy of individuals mentioned in clinical studies, in case histories, and as sources of isolates, do not identify them by their initials, even as part of a strain designa-

tion. Change the initials to numerals or use randomly chosen letters. Do not give hospital unit numbers; if a designation is needed, use only the last two digits of the unit. (Note: established designations of some viruses and cell lines, although they consist of initials, are acceptable [e.g., JC virus, BK virus, and HeLa cells].)

Provision of Requisite Information for Molecular Applications

Authors of manuscripts which contain quantitative real-time PCR applications are encouraged to consult the article concerning minimum information for publication of quantitative real-time PCR experiments (the MIQE guidelines) by Bustin et al. (*Clin. Chem.* 55:611–622, 2009) for guidance as to what information should be considered for inclusion in their submission.

Newly determined nucleotide and/or amino acid sequence data must be deposited and GenBank/ENA/DDBJ accession numbers must be included in the manuscript no later than the modification stage of the review process. It is expected that the sequence data will be released to the public no later than the publication (online posting) date of the accepted manuscript. The accession numbers should be included in a separate paragraph at the end of the Materials and Methods section for full-length papers or at the end of the text for Short-Form papers. If conclusions in a manuscript are based on the analysis of sequences and a GenBank/ENA/DDBJ accession number is not provided at the time of the review, authors should provide the annotated sequence data as supplemental material for information only.

It is expected that when previously published sequence accession numbers are cited in a manuscript, the original citations (e.g., journal articles) will be included in the References section when possible or reasonable.

Authors are also expected to do elementary searches and comparisons of nucleotide and amino acid sequences against the sequences in standard databases (e.g., GenBank) immediately before manuscripts are submitted and again at the proof stage.

Analyses should specify the database, and the date of each analysis should be indicated as, e.g., January 2014. If relevant, the version of the software used should be specified.

See “[Presentation of Nucleic Acid Sequences](#)” for nucleic acid sequence formatting instructions.

The URLs of the databases mentioned above are as follows: DNA Data Bank of Japan (DDBJ), <http://www.ddbj.nig.ac.jp/>; ENA (European Nucleotide Archive), <http://www.ebi.ac.uk/ena/>; and GenBank, National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/>.

Proper Use of Locus Tags as Systematic Identifiers for Genes

To comply with recommendations from the International Nucleotide Sequence Database (INSD) Collaborators and to avoid conflicts in gene identification, researchers should implement the following two fundamental guidelines as standards for utilization of locus tags in genome analysis, annotation, submission, reporting, and publication. (i) Locus tag

prefixes are systematic gene identifiers for all of the replicons of a genome and as such should be associated with a single genome project submission. (ii) New genome projects must be registered with the INSD, and new locus tag prefixes must be assigned in cooperation with the INSD to ensure that they conform to the agreed-upon criteria.

Structural Determinations

Coordinates for new structures of macromolecules must be deposited in the Protein Data Bank and assigned identification codes must be included in the manuscript no later than the modification stage of the review process. It is expected that the coordinates will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to send coordinates with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph at the end of the Materials and Methods section for full-length papers or at the end of the text for Short-Form papers.

The URLs for coordinate deposition are <http://rcsb-deposit.rutgers.edu/> and <http://pdbdep.protein.osaka-u.ac.jp/en/>.

Microarray Data

The entire set of supporting microarray data must be deposited in the appropriate public database (e.g., GEO, ArrayExpress, or CIBEX) and the assigned accession number(s) must be included in the manuscript no later than the modification stage of the review process. It is expected that the data will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to send the relevant data with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph at the end of the Materials and Methods section for full-length papers or at the end of the text for Short-Form papers.

The URLs of the databases mentioned above are as follows: Gene Expression Omnibus (GEO), <http://www.ncbi.nlm.nih.gov/geo/>; ArrayExpress, <http://www.ebi.ac.uk/arrayexpress/>; and Center for Information Biology Gene Expression Database (CIBEX), <http://cibex.nig.ac.jp/data/index.html>.

Culture Deposition

JCM expects authors to deposit important strains in publicly accessible culture collections and to refer to the collections and strain numbers in the text. Since the authenticity of subcultures of culture collection specimens that are distributed by individuals cannot be ensured, authors should indicate laboratory strain designations and donor sources as well as original culture collection identification numbers.

Mycobank

New scientific names of fungi along with key nomenclatural and descriptive material must be deposited in MycoBank (<http://www.mycobank.org>) and the assigned accession num-

ber(s) must be included in the manuscript no later than the modification stage of the review process. It is expected that the data will be released to the public no later than the publication (online posting) date of the accepted manuscript. Authors are encouraged to send the relevant data with their original submission, however, so that reviewers can examine them along with the manuscript. The accession number(s) should be listed in a separate paragraph at the end of the Materials and Methods section for full-length papers or at the end of the text for Short-Form papers.

Supplemental Material

Supplemental material will be peer reviewed along with the manuscript and must be uploaded to the eJournalPress (eJP) peer review system at initial manuscript submission. The decision to publish the material online with the accepted article is made by the editor. It is possible that a manuscript will be accepted but that the supplemental material will not be.

The number of supplemental material files is limited to 10. Supplemental files should be submitted in the following standard formats.

- **Text, figures, tables, and legends** should be included in a single PDF file. All figures and tables should be numbered independently and cited at the relevant point in the manuscript text, e.g. “Fig. S1,” “Fig. S2,” “Table S3,” etc. Do not duplicate data by presenting them in both the text and the figure. Each legend should appear below its corresponding figure or table. The maximum file size is 8 MB. [Please review this sample file for guidance.](#)
- **Data set** (Excel [.xls]) files should include a brief description of how the data are used in the paper. The maximum file size is 8 MB. [Please review this sample file for guidance.](#)
- **Movies** (Audio Video Interleave [.avi], QuickTime [.mov], or MPEG files) should be submitted at the desired reproduction size and length and should be accompanied by a legend. The maximum file size is 20 MB.

Unlike the manuscript, supplemental material will not be edited by the ASM Journals staff and proofs will not be made available. References related to supplemental material only should not be listed in the References section of an article; instead, include them with the supplemental material. Supplemental material will always remain associated with its article and is not subject to any modifications after publication.

Material that has been published previously (print or online) is not acceptable for posting as supplemental material. Instead, the appropriate reference(s) to the original publication should be made in the manuscript.

Copyright for the supplemental material remains with the author, but a license permitting posting by ASM must be signed by the corresponding author. If you are not the copyright owner, you must provide to ASM signed permission from the owner that allows posting of the material, as a supplement to your article, by ASM. You are responsible for including in the supplemental material any copyright notices required by the owner.

See also “[Publication Fees.](#)”

Warranties and Exclusions

Articles published in this journal represent the opinions of the authors and do not necessarily represent the opinions of ASM. ASM does not warrant the fitness or suitability, for any purpose, of any methodology, kit, product, or device described or identified in an article. The use of trade names is for identification purposes only and does not constitute endorsement by ASM.

SUBMISSION, REVIEW, AND PUBLICATION PROCESSES

Submission Process

All submissions to JCM must be made electronically via the eJournalPress (eJP) online submission and peer review system at the following URL: <http://jcm.msubmit.net/cgi-bin/main.plex>. (E-mailed submissions will not be accepted.) First-time users must create an Author account, which may be used for submitting to all ASM journals. Instructions for creating an Author account are available at the above URL via the “help for authors” link, and step-by-step instructions for submitting a manuscript via eJP are also available through the same link on the log-in screen or on the account holder’s Home page. Information on file types acceptable for electronic submission can be found under the Files heading in the help for authors screen.

Review Process

All manuscripts are considered to be confidential and are reviewed by the editors, members of the editorial board, or qualified *ad hoc* reviewers.

To expedite the review process, authors must recommend at least three editorial board members who have expertise in the field, who are not members of their institution(s), who have not recently been associated with their laboratory(ies), and who could not otherwise be considered to pose a conflict of interest regarding the submitted manuscript.

Copies of in-press and submitted manuscripts that are important for judgment of the present manuscript should be included as supplemental material for information only to facilitate the review.

When a manuscript is submitted to the journal, it is given a control number (e.g., JCM00123-14) and assigned to one of the editors. (**Always refer to this control number in communications with the editor and the Journals Department.**) It is the responsibility of the corresponding author to inform the coauthors of the manuscript’s status throughout the submission, review, and publication processes. The reviewers operate under strict guidelines set forth in “Guidelines for Reviewers” (<http://www.journals.asm.org/site/misc/reviewguide.xhtml>) and are expected to complete their reviews expeditiously.

The corresponding author is notified, generally within 4 to 6 weeks after submission, of the editor’s decision to accept, reject, or require modification. When modification is requested, the corresponding author must either submit the modified version within 2 months or withdraw the manuscript. A point-by-point response to the reviews must be loaded as a separate

file (identified as such), and a compare copy of the manuscript (without figures) should be included as a Marked Up Manuscript if the editor requested one.

Manuscripts that have been rejected, or withdrawn after being returned for modification, may be resubmitted to the same ASM journal if the major criticisms have been addressed. A manuscript rejected by one ASM journal on scientific grounds or on the basis of its general suitability for publication is considered rejected by all other ASM journals; however, a manuscript rejected solely on the basis of scope may be “resubmitted” to a more appropriate ASM journal.

For all resubmissions (to the same or a different journal, irrespective of the extent of the revisions and irrespective of the amount of time between rejection and resubmission), the cover letter must state that the manuscript is a resubmission, and the former manuscript control number must be provided. A point-by-point response to the review(s) must be loaded as a separate file (identified as such), and a compare copy of the revised manuscript showing all changes must be included as a Marked Up Manuscript. Manuscripts resubmitted to the same journal are normally handled by the original editor.

Rejected manuscripts may be resubmitted only once unless permission has been obtained from the original editor or from the editor in chief.

Notification of Acceptance

When an editor has decided that a manuscript is acceptable for publication on the basis of scientific merit, the author and the Journals Department are notified. A PDF version of the accepted manuscript is posted online as soon as possible (see “JCM Accepts”).

The text files undergo an automated preediting, cleanup, and tagging process specific to the particular article type, and the illustrations are examined. If all files have been prepared according to the criteria set forth in these Instructions and those in the eJP online manuscript submission system, the acceptance procedure will be completed successfully. If there are problems that would cause extensive corrections to be made at the copyediting stage or if the files are not acceptable for production, ASM Journals staff will contact the corresponding author. Once all the material intended for publication has been determined to be adequate, the manuscript is scheduled for the next available issue. The editorial staff of the ASM Journals Department completes the editing of the manuscript to bring it into conformity with prescribed standards.

JCM Accepts

For its primary-research journals, ASM posts online PDF versions of manuscripts that have been peer reviewed and accepted but not yet copyedited. This feature is called “[journal acronym] Accepts” (e.g., JCM Accepts) and is accessible from the Journals website. The manuscripts are published online as soon as possible after acceptance, on a weekly basis, before the copyedited, typeset articles are published. They are posted “as is” (i.e., as submitted by the authors at the modification stage) and do not reflect ASM editorial changes. No corrections/changes to the PDF manuscripts are accepted. Accordingly, there likely will be differences between the JCM Accepts man-

uscripts and the final, typeset articles. The manuscripts remain listed on the JCM Accepts page until the final, typeset articles are posted. At that point, the manuscripts are removed from the JCM Accepts page. The manuscripts are under subscription access control until 6 months after the typeset articles are posted, when free access is provided to everyone (subject to the applicable ASM license terms and conditions). Supplemental material intended, and accepted, for publication is not posted until publication of the final, typeset article.

The ASM embargo policy allows a press release to be issued as soon as the ahead-of-print manuscript is posted on the JCM Accepts page. To be notified as soon as your manuscript is posted, please sign up for e-Alerts at <http://jcm.asm.org/cgi/alerts>.

Instructions on how to cite such manuscripts may be found in “References.”

Page Proofs

Page proofs, together with a query sheet and instructions for handling proofs, will be made available to the corresponding author electronically. Queries must be answered on the query page, and any changes related to the queries must be indicated on the proofs. Note that the copy editor does not query at every instance where a change has been made. Queries are written only to request necessary information or clarification of an unclear passage or to draw attention to edits that may have altered the sense. It is the author’s responsibility to read the entire text, tables, and figure legends, not just items queried. Corrected proofs must be returned within 48 hours after notification of availability.

The proof stage is not the time to make extensive corrections, additions, or deletions. Figures as they appear in the proofs are for validation of content and placement, not quality of reproduction or color accuracy. Print output of figures in the PDF page proofs will be of lower quality than the same figures viewed on a monitor. Please avoid making changes to figures based on quality of color or reproduction in proof.

Important new information that has become available between acceptance of the manuscript and receipt of the proofs may be inserted as an addendum in proof with the permission of the editor. If references to unpublished data or personal communications are added, it is expected that written assurance granting permission for the citation will be included. Limit changes to correction of spelling errors, incorrect data, and grammatical errors and updated information for references to articles that have been submitted or are in press. If URLs have been provided in the article, recheck the sites to ensure that the addresses are still accurate and the material that you expect the reader to find is indeed there.

Questions about late proofs and problems with the proofs should be directed to the ASM Journals Department (e-mail, cbrown@asmusa.org; telephone, 202-942-9384).

PDF Files

A corresponding author who has included an e-mail address in his/her “corresponding author” footnote will have limited access (10 downloads, total) to the PDF file of his/her published article. An e-mail alert will automatically be sent to him/her on

the day the issue is posted. It will provide a URL, which will be required to obtain access, and instructions. An article may be viewed, printed, or stored, provided that it is for the author's own use.

Should coauthors or colleagues be interested in viewing the paper for their own use, the corresponding author may provide them with the URL; a copy of the article may not be forwarded electronically. However, they must be made aware of the terms and conditions of the ASM copyright. (For details, go to <http://www.journals.asm.org/site/misc/terms.xhtml>.) Note that each such download will count toward the corresponding author's total of 10. After 10 downloads, access will be denied and can be obtained only through a subscription to the journal (either individual or institutional) or after the standard access control has been lifted (i.e., 6 months after publication).

Publication Fees

Page charges. Authors whose research was supported by grants, special funds (including departmental and institutional), or contracts (including governmental) or whose research was done as part of their official duties (government or corporate, etc.) are required to pay page charges (based on the number of typeset pages, including illustrations, in the article). Corresponding authors of articles accepted for publication will receive an e-mail notifying them how to pay page and any other applicable publication charges (see below).

For a **corresponding author who is an active member of ASM at the Contributing or Premium level**, page charges are \$67 per page for the first eight pages and \$125 per page for each page in excess of eight (subject to change without notice).

For a **nonmember or Supporting member corresponding author**, page charges are \$135 per page for the first eight pages and \$250 for each page in excess of eight (subject to change without notice). Nonmember corresponding authors or Supporting members may [join ASM and renew or upgrade membership online](#) to obtain discounts on publication fees.

If the research was not supported by any of the means described above, a request to waive the charges may be sent to the Journals Department, ASM, 1752 N St., N.W., Washington, DC 20036-2904, USA (fax, 202-942-9355; e-mail, cbrown@asmusa.org [after acceptance of the manuscript]). The request must include the manuscript control number assigned by ASM and indicate how the work was supported. Waivers apply only to page charges; responsibility for color charges and other publication fees remains with the author.

Minireviews, Commentaries, Photo Quizzes, and Comment Letters to the Editor are not subject to page charges. New-Data Letters to the Editor are subject to page charges.

Color charges. The cost of publishing in color must be borne by the author.

For a **corresponding author who is an active member of ASM at the Contributing or Premium level**, color charges are \$170 per color figure (subject to change without notice).

For a **nonmember or Supporting member corresponding author**, color charges are \$375 per color figure (subject to change without notice). Nonmember corresponding authors or Supporting members may [join ASM and renew or upgrade membership online](#) to obtain discounts on publication fees.

Minireviews, Commentaries, Photo Quizzes, and Comment Letters to the Editor are not subject to color charges. New-Data Letters to the Editor are subject to color charges.

Author reprints and eprints. Reprints (in multiples of 100) and eprints (downloadable PDFs) may be purchased by all coauthors. In addition to the 10 free published PDF files mentioned above, the corresponding authors of Minireviews may receive 100 free eprints of their contribution and the corresponding authors of Commentaries may receive 50 free eprints. Instructions for ordering gratis or additional reprints and eprints can be found in the billing notification e-mail sent to all corresponding authors. To order reprints postpublication, please follow the instructions on the Author Reprint Order Form. Please contact cjsreprints@cadmus.com with any questions.

Supplemental material fee. Authors are charged a flat fee for posting supplemental material as an adjunct to their published article. (Exception: no fee is charged for supplemental material associated with Minireviews or Commentaries.)

For a **corresponding author who is an active member of ASM at the Contributing or Premium level**, the supplemental material fee is \$190. For a **nonmember or Supporting member corresponding author**, the supplemental material fee is \$285. Nonmember corresponding authors or Supporting members may [join ASM and renew or upgrade membership online](#) to obtain discounts on publication fees.

Optional open access fee. Author-paid optional open access (OOA) is now available for all article types. For a **corresponding author who is an active member of ASM at the Contributing or Premium level**, the OOA fee is \$2,000. For a **nonmember or Supporting member corresponding author**, the OOA fee is \$3,000. Nonmember corresponding authors or Supporting members may [join ASM and renew or upgrade membership online](#) to obtain discounts on publication fees. These fees are in addition to any page charges, color charges, or supplemental material charges and permit immediate public access to both the preliminary "Accepts" version and the copy-edited, typeset version published in the online journal. This option includes immediate open access provided through NIH's PubMed Central repository; all primary research published in ASM journals is freely available through PubMed Central 6 months after publication.

When funds from the Wellcome Trust or Research Councils UK are used to pay an article open access fee, the article will be published under the [Creative Commons Attribution license \(CC-BY\)](#) in accordance with the funding organization's open access policies. Authors will be required to notify ASM and complete the Author Warranty and Provisional License to Publish at the time of submission.

ORGANIZATION AND FORMAT

Editorial Style

The editorial style of ASM journals conforms to the *ASM Style Manual for Journals* (American Society for Microbiology,

2014, in-house document) and *How To Write and Publish a Scientific Paper*, 7th ed. (Greenwood, Santa Barbara, CA, 2011), as interpreted and modified by the editors and the ASM Journals Department.

The editors and the Journals Department reserve the privilege of editing manuscripts to conform with the stylistic conventions set forth in the aforesaid publications and in these Instructions.

On receipt at ASM, an accepted manuscript undergoes an automated preediting, cleanup, and tagging process specific to the particular article type. To optimize this process, manuscripts must be supplied in the correct format and with the appropriate sections and headings.

Type every portion of the manuscript double-spaced (a minimum of 6 mm between lines), including figure legends, table footnotes, and References, and number all pages in sequence, including the abstract, figure legends, and tables. Place the last two items after the References section. Manuscript pages must have continuous line numbers and page numbers. Manuscripts without line and page numbers will be returned to authors for provision of this information prior to processing. The font size should be no smaller than 12 points. It is recommended that the following sets of characters be easily distinguishable in the manuscript: the numeral zero (0) and the letter “oh” (O); the numeral one (1), the letter “el” (l), and the letter “eye” (I); and a multiplication sign (×) and the letter “ex” (x). Do not create symbols as graphics or use special fonts that are external to your word processing program; use the “insert symbol” function. Set the page size to 8.5 by 11 inches (ca. 21.6 by 28 cm). Italicize any words that should appear in italics, and indicate paragraph lead-ins in boldface type.

Manuscripts may be editorially rejected, without review, on the basis of poor English or lack of conformity to the standards set forth in these Instructions.

Authors who are unsure of proper English usage should have their manuscripts checked by someone proficient in the English language or engage a professional language editing service for help.

Manuscript Submission Checklist

- Double-space all text, including references and figure legends.
- Number pages.
- Number lines continuously.
- Present statistical treatment of data where appropriate.
- Format references in ASM style.
- Provide accession numbers for all newly published sequences in a dedicated paragraph, and if a sequence or sequence alignment important for evaluation of the manuscript is not yet available, provide the information as supplemental material for information only or make the material available on a website for access by the editor and reviewers.
- Confirm that genetic and chemical nomenclature conforms to instructions.
- Include as supplemental material for information only in-press and submitted manuscripts that are important for judgment of the present manuscript.

Full-Length Papers

Full-length papers include the elements described in this section.

Title, running title, and byline. Each manuscript should present the results of an independent, cohesive study; thus, numbered series titles are not permitted. Exercise care in composing a title. Avoid the main title/subtitle arrangement, complete sentences, and unnecessary articles. On the title page include the title, the running title (not to exceed 54 characters and spaces), the name of each author, all authors’ affiliations at the time the work was performed, and a footnote indicating the present address(es) of any author(s) no longer at the institution where the work was performed. Place a number sign (#) after the name of the author to whom inquiries regarding the paper should be directed (see “[Correspondent footnote](#),” below). [Please review this sample title page for guidance.](#)

Study group in byline. A study group, surveillance team, working group, consortium, or the like (e.g., the Active Bacterial Core Surveillance Team) may be listed as a coauthor in the byline if its contributing members satisfy the requirements for authorship and accountability as described in these Instructions. The names (and institutional affiliations if desired) of the contributing members may be given as a separate paragraph in Acknowledgments.

If the contributing members of the group associated with the work do not fulfill the criteria of substantial contribution to and responsibility for the paper, the group may not be listed in the author byline. Instead, it and the names of its contributing members may be listed in the Acknowledgments section.

Correspondent footnote. The e-mail address for the corresponding author should be included on the title page of the manuscript. This information will be published in the article as a footnote to facilitate communication and will be used to notify the corresponding author of the availability of proofs and, later, of the PDF file of the published article. No more than two authors may be designated corresponding authors.

Abstract. Limit the abstract to 250 words or fewer and concisely summarize the basic content of the paper without presenting extensive experimental details. Avoid abbreviations and references, and do not include diagrams. When it is essential to include a reference, use the same format as shown for the References section but omit the article title. Conclude the abstract with a summary statement. Because the abstract will be published separately by abstracting services, it must be complete and understandable without reference to the text.

Introduction. The introduction should supply sufficient background information to allow the reader to understand and evaluate the results of the present study without referring to previous publications on the topic. The introduction should also provide the hypothesis that was addressed or the rationale for the present study. Choose references carefully to provide the most salient background rather than an exhaustive review of the topic.

Case Report. The Case Report section, placed after the introduction and before Materials and Methods, is optional and gives relevant clinical information about one or more patients while being incidental to the rest of the paper. (If the Case Report constitutes the entire article, the paper must be presented in Case Report format [see “Case Reports,” below], which differs from that used for a full-length text or a Short-Form paper.)

Materials and Methods. The Materials and Methods section must include sufficient technical information to allow the experiments to be repeated. The sources of all media (i.e., name and location of manufacturer) or components of a new formulation must be provided. When centrifugation conditions are critical, give enough information to enable another investigator to repeat the procedure: make of centrifuge, model of rotor, temperature, time at maximum speed, and centrifugal force ($\times g$ rather than revolutions per minute). For commonly used materials and methods (e.g., media and protein concentration determinations), a simple reference or specifically recommended product or procedure is sufficient. If several alternative methods are commonly used, it is helpful to identify the method briefly as well as to cite the reference. For example, it is preferable to state “cells were broken by ultrasonic treatment as previously described (9)” rather than to state “cells were broken as previously described (9).” This allows the reader to assess the method without constant reference to previous publications. Describe new methods completely, and give sources of unusual chemicals, reagents, equipment, or microbial strains. When large numbers of microbial strains or mutants are used in a study, include tables identifying the immediate sources (i.e., sources from whom the strains were obtained) and properties of the strains, mutants, bacteriophages, and plasmids, etc.

A method or strain, etc., used in only one of several experiments reported in the paper may be described in the Results section or very briefly (one or two sentences) in a table footnote or figure legend. It is expected that the sources from whom the strains were obtained will be identified.

Results. In the Results section, include the rationale or design of the experiments as well as the results; reserve extensive interpretation of the results for the Discussion section. Present the results as concisely as possible in one of the following: text, table(s), or figure(s). Avoid extensive use of graphs to present data which might be more concisely presented in the text or tables. For example, except in unusual cases, double-reciprocal plots used to determine apparent K_m values should not be presented as graphs; instead, the values should be stated in the text. Similarly, graphs illustrating other methods commonly used to derive kinetic or physical constants (e.g., reduced-viscosity plots and plots used to determine sedimentation velocity) need not be shown except in unusual circumstances. All tabular data must be accompanied by either standard deviation values or standard errors of the means. The number of replicate determinations (or animals) used for making such calculations must also be included. All statements concerning the significance of the differences observed should be accompanied by probability values given in parentheses. The statistical

procedure used should be stated in Materials and Methods. Limit illustrations (particularly photomicrographs and electron micrographs) to those that are absolutely necessary to show the experimental findings. Number figures and tables in the order in which they are cited in the text, and be sure to cite all figures and tables.

Discussion. The Discussion section should provide an interpretation of the results in relation to previously published work and to the experimental system at hand. It must not contain extensive repetition of the Results section or reiteration of the introduction. In short papers, the Results and Discussion sections may be combined.

Acknowledgments. The source of any financial support received for the work being published must be indicated in the Acknowledgments section. (It will be assumed that the absence of such an acknowledgment is a statement by the authors that no support was received.) The usual format is as follows: “This work was supported by Public Health Service grant CA-01234 from the National Cancer Institute.”

Recognition of personal assistance should be given as a separate paragraph, as should any statements disclaiming endorsement or approval of the views reflected in the paper or of a product mentioned therein.

Appendixes. Appendixes that contain additional material to aid the reader are permitted. Titles, authors, and reference sections that are distinct from those of the primary article are not allowed. If it is not feasible to list the author(s) of the appendix in the byline or the Acknowledgments section of the primary article, rewrite the appendix so that it can be considered for publication as an independent article, either full-length paper or Short-Form style. Equations, tables, and figures should be labeled with the letter “A” preceding the numeral to distinguish them from those cited in the main body of the text.

References. In the reference list, references are numbered in the order in which they are cited in the article (citation-sequence reference system); ASM no longer uses the citation-name system with an alphabetized reference list. In the text, references are cited parenthetically by number in sequential order. Data that are not published or not peer reviewed are simply cited parenthetically in the text (see section ii below).

(i) **References listed in the References section.** The following types of references must be listed in the References section:

- Journal articles (both print and online)
- Books (both print and online)
- Book chapters (book title is required)
- Patents
- Theses and dissertations
- Published conference proceedings
- Meeting abstracts (from published abstract books or journal supplements)
- Letters (to the editor)
- Company publications

- In-press journal articles, books, and book chapters (publication title is required)

Provide the names of all the authors and/or editors for each reference; names should not be abbreviated with “et al.”

Since title and byline information that is downloaded from PubMed does not always show accents, italics, or special characters, authors should refer to the PDF files or hard-copy versions of the articles and incorporate the necessary corrections in the submitted manuscript. Abbreviate journal names according to the PubMed Journals Database (National Library of Medicine, National Institutes of Health; available at <http://www.ncbi.nlm.nih.gov/nlmcatalog/journals>), the primary source for ASM style.

Follow the styles shown in the examples below for print references.

1. Caserta E, Haemig HAH, Manias DA, Tomsic J, Grundy FJ, Henkin TM, Dunny GM. 2012. *In vivo* and *in vitro* analyses of regulation of the pheromone-responsive *prgQ* promoter by the PrgX pheromone receptor protein. *J. Bacteriol.* **194**:3386–3394.
2. Falagas ME, Kasiakou SK. 2006. Use of international units when dosing colistin will help decrease confusion related to various formulations of the drug around the world. *Antimicrob. Agents Chemother.* **50**:2274–2275. (Letter.) {“Letter” or “Letter to the editor” is allowed but not required at the end of such an entry.}
3. Cox CS, Brown BR, Smith JC. *J. Gen. Genet.*, in press.* {Article title is optional; journal title is mandatory.}
4. da Costa MS, Nobre MF, Rainey FA. 2001. Genus I. *Thermus* Brock and Freeze 1969, 295, ^{AL} emend. Nobre, Trüper and da Costa 1996b, 605, p 404–414. In Boone DR, Castenholz RW, Garrity GM (ed), *Bergey’s manual of systematic bacteriology*, 2nd ed, vol 1. Springer, New York, NY.
5. 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.}
6. Forman MS, Valsamakis A. 2011. Specimen collection, transport, and processing: virology, p 1276–1288. In Versalovic J, Carroll KC, Jorgensen JH, Funke G, Landry ML, Warnock DW (ed), *Manual of clinical microbiology*, 10th ed, vol 2. ASM Press, Washington, DC.
7. Fitzgerald G, Shaw D. In Waters AE (ed), *Clinical microbiology*, in press. EFH Publishing Co, Boston, MA.* {Chapter title is optional.}
8. 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.}
9. Carlson E. 2013. Selective penicillin-binding protein imaging probes reveal substructure in bacterial cell division, p 59. Final Program 113th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC. {Abstract title is optional.}
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. 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.
12. O’Malley DR. 1998. Ph.D. thesis. University of California, Los Angeles, CA. {Title is optional.}
13. 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.}
14. Elder BL, Sharp SE. 2003. Cumitech 39, Competency assessment in the clinical laboratory. Coordinating ed, Sharp SE. ASM Press, Washington, DC.

*A reference to an in-press ASM publication should state the control number (e.g., JCM00123-14) if it is a journal article or the name of the publication if it is a book.

Online-only references must provide essentially the same information that print references do. For online journal articles, posting or revision dates may replace the year of publication; a DOI (preferred) or URL is required for articles with nontraditional page numbers or electronic article identifiers.

1. Bina XR, Taylor DL, Vikram A, Ante VM, Bina JE. 2013. *Vibrio cholerae* ToxR downregulates virulence factor production in response to cyclo(Phe-Pro). *mBio* **4**(5):e00366-13. doi:10.1128/mBio.00366-13.
2. Winnick S, Lucas DO, Hartman AL, Toll D. 2005. How do you improve compliance? *Pediatrics* **115**:e718–e724. doi:10.1542/peds.2004-1133.
3. Dionne MS, Schneider DS. 2002. Screening the fruitfly immune system. *Genome Biol.* **3**:reviews1010-reviews1010.2. doi:10.1186/gb-2002-3-4-reviews1010.
4. Gregory ST. 2 September 2009. Chapter 2.5.4, Structural basis for the decoding mechanism. In Böck A, et al (ed), *EcoSal—Escherichia coli and Salmonella: cellular and molecular biology*. ASM Press, Washington, DC. doi:10.1128/ecosal.2.5.4. {Note that each chapter has its own posting date.}

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 polyleucine transmembrane helices. *Proc. Natl. Acad. Sci. U. S. A.* doi:10.1073/pnas.041593698.

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

- 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
- Computer software, databases, and 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.}*
- ... available in the GenBank database (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>).
- ... using ABC software (version 2.2; Department of Microbiology, State University [<http://www.state.micro.edu>]).

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. Satheskumar, 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(5): 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 references 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 papers. However, Short-Form papers should contain firm data; observations alone are not acceptable. Submit Short-Form papers in the same way as full-length papers. They receive the same review, they are not published more rapidly than full-length papers, 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 full-length paper. Each Short-Form paper must have an abstract of no more than 50 words. Do not use section headings in the body of the Short Form; 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 full-length papers. The References section is identical to that of full-length papers.

Minireviews

Minireviews are expected to be focused discussions of defined topics relevant to clinical microbiologists. In general, they are to be submitted only following invitation by the editor in chief of JCM. Unsolicited Minireviews are discouraged. A topical outline should be provided to the editor in chief for approval prior to submission of the completed Minireview manuscript in the eJP online manuscript submission and peer review system.

Minireviews are not expected to be comprehensive reviews of the literature but rather focused discussions of specific topics. A standard title page should be provided. This is followed by an abstract of 100 words or less and then the text of the Minireview, which should not exceed 12 double-spaced manuscript pages in length, exclusive of tables, figures, photographs, and references. Up to three tables, figures, or photographs, total, may be included. References should be limited to no more than 30. Minireviews will be reviewed by two JCM editors, with the aim of expedited processing. In general, it is hoped that, barring the necessity of major revisions, accepted Minireviews will appear in print within 3 months of their submission and online ahead of print 6 to 8 weeks earlier.

Author bio. A short biographical sketch and photograph of the **one** author most responsible for the minireview should be submitted along with the initial version of the manuscript. These will be published at the end of the article.

- The text limit is 150 words 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 area, as well as a brief review of your scholarly interests and record of publication. In addition, please list pertinent significant awards you have received.
- The photo should be a recent black-and-white head shot of passport size. It will be reduced to approximately 1.125 inches wide by 1.375 inches high. The photo 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 photo with your manuscript in the submission and review system. Include the biographical text immediately **after** the References section of your manuscript, in the same file. It should be labeled with the heading “Biosketch.” Upload the head shot photograph in the submission system as a “Minireview Bio Photo”; **include the author’s name or enough of it for identification in the 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 JCM 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 four printed pages, and the format is like that of a Minireview (see above) except that the abstract is limited to 75 words.

Point-Counterpoint

Point-Counterpoint is a feature of JCM in which two experts present opposing views on a contemporary issue in the laboratory diagnosis of infectious diseases. This feature will be the lead article in the issue of JCM in which it appears. Participation as an author of a Point-Counterpoint feature is by invitation only.

A JCM editor will write a brief introductory piece of approximately 200 words outlining why a specific issue is important and then present the issue in the form of a question. The two experts will then each write a commentary, no more than 1,000 words in length, in which they present evidence in support of either the pro or con view. One table or one figure may be included. Since these discussions will be evidence based, authors may also cite up to 10 references. Unpublished or in-

press data which reflect the current practice in their laboratory may be used but should not be the sole basis for their position.

Authors should send commentaries directly back to the JCM editor within 30 days of receipt of the introductory statement. Following receipt of both the pro and con commentaries, the editor will review the submissions and may return them to the author(s) with comments and/or suggested revisions. If revisions are required, the author(s) will have 14 days to craft a revised commentary, which will be sent directly back to the editor. Upon receipt of final commentaries, the JCM editor will write a brief summary consisting of no more than six one-sentence bullet points, outlining where the experts agree (no more than three points) and disagree (no more than three points). The JCM editor will then upload the introduction, both commentaries, and the summary in eJP.

Case Reports

While a full-length article or a Short-Form paper may contain a case report section when the report is incidental to the rest of the paper, a specific Case Report format must be used when the report constitutes the entire article.

A Case Report must include an abstract of no more than 50 words. The text starts with presentation of the case under the section heading “Case Report”; there is no introductory text before the Case Report heading. After the case is presented, the rest of the text follows in a separate section after a ruled line to separate the sections. No separate head is used for this short discussion section, but paragraph lead-ins are permitted. The total number of tables and figures (combined) must not exceed 3. For an example of a correctly formatted Case Report, see *J. Clin. Microbiol.* **39**:1678–1679, 2001.

Photo Quiz

A Photo Quiz submission should present the findings of some relevant, interesting, and new observation pertinent to the practice of clinical microbiology in which a photograph is particularly useful in conveying important information **and** where the observation can serve as the basis for both a question and an answer. The photograph may be of a micrograph, some other laboratory material, a clinical lesion, or the results of an imaging study.

A Photo Quiz consists of two parts: (i) a case presentation featuring a photograph depicting some unusual and/or informative finding in clinical microbiology and (ii) an answer to the quiz. The case presentation and the answer must be submitted as two separate articles. Note that authors and affiliations are listed below the title.

Photo Quiz case presentation. The text in the Photo Quiz case presentation should be limited to 200 to 300 words. The header for the case presentation should read “Photo Quiz.”

Please include a photograph about 39 picas (6.5 inches) wide and 28 picas (4.625 inches) high. Since photos appearing with published Photo Quizzes appear on the cover of the journal, a high-resolution TIFF or EPS file is preferred. A short legend for the photo must be provided, and the photo must be cited in the case presentation. Refer to a recently published Photo Quiz for correct formatting.

Answer to Photo Quiz. The text of the answer to the Photo Quiz should also be limited to 200 to 300 words. The header to the answer should read “Answer to Photo Quiz.” Four to six references may be cited at the end of the Photo Quiz answer.

Submission. The Photo Quiz case presentation should be submitted in the “Photo Quiz” manuscript category. The Photo Quiz answer should be submitted in the “Photo Quiz Answer” manuscript category.

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 publish-ahead-of-print manuscripts) 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 full-length papers 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 drop-down list 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.

Fast-Track Communications

The Fast-Track route is intended for accelerated review of short communications that are of significant interest to clinical microbiologists. Manuscripts are limited to 750 words, one figure, one table, and 10 or fewer references. The format should be the same as that of a New-Data Letter (see “[Letters to the Editor](#),” above). Fast-Track articles should be submitted via the eJP online manuscript submission and peer review system.

A Fast-Track submission is subject to approval as such by the editor in chief. If approved for the Fast-Track route, the manuscript will be assigned to an appropriate JCM editor and reviewed, according to the same standards applied for traditional manuscripts, within 1 week. If accepted, the manuscript will be scheduled for the next available issue and edited. An

acceptance letter and copyright agreement will be mailed to the corresponding author. Proofs will be made available electronically as for regular articles.

A Fast-Track submission that is not approved for the Fast-Track route will be handled as a New-Data Letter according to normal procedures.

Errata

The Erratum section provides 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

The Author Correction section provides 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 for information only (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 for information only (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.

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 distinguish 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, but the cost of printing in color must be borne by the author. Suggestions about how to reduce costs and 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 figure to assist review. At the modification stage, production-quality digital files must be provided. The legends will be copy-edited and typeset for final publication 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](#).” Halftone images (those with various densities or shades) must be grayscale, not bitmap. JCM 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, im-

properly 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.

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

Minimum resolution. It is extremely important that a high enough 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**; that is, the image uploaded should be 100% of its print dimensions 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 width for a 1-column figure: 20.6 picas (ca. 8.7 cm)
- Maximum width for a 2-column figure: 42 picas (ca. 17.8 cm)
- Minimum width for a 2-column figure: 26 picas (11.1 cm)
- Maximum height for a standard figure: 54.7 picas (ca. 23.2 cm)
- Maximum height for an oversized figure (no running title); 57.4 picas (ca. 24.3 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, where a non-proportional (monospace) font is required. All fonts other than these must be converted to paths (or outlines) in the application with which they were created. For proper font use in PowerPoint images, refer to the Cadmus digital art website, http://art.cadmus.com/da/instructions/ppt_disclaimer.jsp.

Color illustrations. Color costs must be borne by the author. See “Publication Fees.” All figures submitted in color will be processed as color. Adherence to the following guidelines will help to minimize costs and to ensure color reproduction that is as accurate as possible.

The final online version is considered the version of record for JCM 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 the print version (and reprints), ASM’s print provider will automatically create CMYK versions of color illustrations from the supplied RGB versions. Color in the print journal 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. Keep in mind that the journal is published both in print and online and that the same electronic files submitted by the authors are used to produce both.

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) No type should be 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 appropriate *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, 2011); an abbreviated list is available at <http://old.iupac.org/reports/1993/homann/index.html>.

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 of 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 sequence 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 reported in a legend only if the discussion 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 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.

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*, 15th ed. (RSC Books, Cambridge, UK, 2013), is also an excellent source. For biochemical terminology, including abbreviations and symbols, consult *Biochemical Nomenclature and Related Documents* (Portland Press, London, United Kingdom, 1992) available at <http://www.chem.qmul.ac.uk/iupac/bibliog/white.html>, and the instructions to authors of the *Journal of Biological Chemistry* and the *Archives of Biochemistry and Biophysics*.

Do not express molecular weight in daltons; molecular weight is a unitless ratio. Molecular mass is expressed in daltons.

For enzymes, use the recommended (trivial) name 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.chem.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 STREND A Commission for information required for adequate description of experimental conditions and for reporting enzyme activity data (<http://www.beilstein-institut.de/en/projekte/strenda/guidelines/>).

For nomenclature of restriction enzymes, DNA methyltransferases, homing endonucleases, and their genes, refer to the article by Roberts et al. (*Nucleic Acids Res.* **31**:1805–1812, 2003).

Drugs

Whenever possible, use generic names of drugs; the use of trade names is not permitted.

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 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.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089>). 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 (<http://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.

For guidelines regarding new names and descriptions of new genera and species, see the articles by Tindall (*Int. J. Syst. Bacteriol.* **49**:1309–1312, 1999) and Stackebrandt et al. (*Int. J. Syst. Evol. Microbiol.* **52**:1043–1047, 2002). To validate new names and/or combinations, authors must submit three copies of their published article to the *International Journal of Systematic and Evolutionary Microbiology*.

It is recommended that a strain be deposited in at least two recognized culture collections in different countries when that strain is necessary for the description of a new taxon (*Int. J. Syst. Evol. Microbiol.* **50**:2239–2244, 2000).

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 (<http://www.ictvonline.org/index.asp>). 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 italicized 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. Use the recommendations of Demerec et al. (*Genetics* **54**:61–64, 1966) as a guide to the use of these terms. If your manuscript contains information including genetic nomenclature, please refer to the Instructions to Authors of the *Journal of Bacteriology*.

“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.

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.

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.

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.

Eukaryotes. FlyBase (<http://flybase.org/>) is the genetic nomenclature authority for *Drosophila melanogaster*. WormBase (<http://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.aspergillus.org.uk/indexhome.htm?secure/sequence_info/nomenclature.htm 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, respec-

tively. For more information about the genetic nomenclature of eukaryotes, see the Instructions to Authors for *Eukaryotic Cell* and *Molecular and Cellular Biology*.

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 for 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)	tRNA (transfer RNA)
cDNA (complementary DNA)	AMP, ADP, ATP, dAMP,
RNA (ribonucleic acid)	ddATP, and GTP, etc. (for the
cRNA (complementary RNA)	respective 5' phosphates
RNase (ribonuclease)	of adenosine and other
DNase (deoxyribonuclease)	nucleosides) (add 2'-,
rRNA (ribosomal RNA)	3'-, or 5'- when needed for
mRNA (messenger RNA)	contrast)

ATPase and dGTPase, etc.
(adenosine triphosphatase and deoxyguanosine triphosphatase, etc.)
NAD (nicotinamide adenine dinucleotide)
NAD⁺ (nicotinamide adenine dinucleotide, oxidized)
NADH (nicotinamide adenine dinucleotide, reduced)
NADP (nicotinamide adenine dinucleotide phosphate)
NADPH (nicotinamide adenine dinucleotide phosphate, reduced)
NADP⁺ (nicotinamide adenine dinucleotide phosphate, oxidized)
poly(A) and poly(dT), etc. (polyadenylic acid and polydeoxythymidylic acid, etc.)

oligo(dT), etc. (oligodeoxythymidylic acid, etc.)
UV (ultraviolet)
PFU (plaque-forming units)
CFU (colony-forming units)
MIC (minimal inhibitory concentration)
Tris (tris[hydroxymethyl]aminomethane)
DEAE (diethylaminoethyl)
EDTA (ethylenediamine-tetraacetic acid)
EGTA (ethylene glycol-bis[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid)
HEPES (N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid)
PCR (polymerase chain reaction)
AIDS (acquired immunodeficiency syndrome)

cephalexin (LEX)
cephalothin (CEF)
cephapirin (HAP)
cephradine (RAD)
chloramphenicol (CHL)
cinoxacin (CIN)
ciprofloxacin (CIP)
clarithromycin (CLR)
clinafloxacin (CLX)
clindamycin (CLI)
daptomycin (DAP)
dicloxacillin (DCX)
dirithromycin (DTM)
doxycycline (DOX)
enoxacin (ENX)
erythromycin (ERY)
floxacin (FLE)
fosfomicin (FOF)
gatifloxacin (GAT)
gentamicin (GEN)
grepafloxacin (GRX)
imipenem (IPM)
kanamycin (KAN)
levofloxacin (LVX)
linezolid (LZD)
lomefloxacin (LOM)
loracarbef (LOR)
meropenem (MEM)
methicillin (MET)
mezlocillin (MEZ)
minocycline (MIN)

moxalactam (MOX)
moxifloxacin (MXF)
nafcillin (NAF)
nalidixic acid (NAL)
netilmicin (NET)
nitrofurantoin (NIT)
norfloxacin (NOR)
ofloxacin (OFX)
oxacillin (OXA)
penicillin (PEN)
piperacillin (PIP)
piperacillin-tazobactam (TZP)
quinupristin-dalfopristin (Synercid) (Q-D)
rifabutin (RFB)
rifampin (RIF)
rifapentine (RFP)
sparfloxacin (SPX)
spectinomycin (SPT)
streptomycin (STR)
teicoplanin (TEC)
telithromycin (TEL)
tetracycline (TET)
ticarcillin (TIC)
ticarcillin-clavulanic acid (TIM)
tobramycin (TOB)
trimethoprim (TMP)
trimethoprim-sulfamethoxazole (SXT)
trovafloxacin (TVA)
vancomycin (VAN)

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

The following abbreviations should be used without definition in tables:

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

Drugs. Should an author decide to abbreviate the names of antimicrobial agents in a manuscript, the following standard abbreviations are strongly recommended.

Antibacterial agents. Use the indicated abbreviations for the following antibacterial agents.

amikacin (AMK)	cefetamet (FET)
amoxicillin (AMX)	cefixime (CFM)
amoxicillin-clavulanic acid (AMC)	cefmetazole (CMZ)
ampicillin (AMP)	cefonicid (CID)
ampicillin-sulbactam (SAM)	cefoperazone (CFP)
azithromycin (AZM)	cefotaxime (CTX)
azlocillin (AZL)	cefotetan (CTT)
aztreonam (ATM)	cefoxitin (FOX)
carbenicillin (CAR)	cefpodoxime (CPD)
cefaclor (CEC)	cefprozil (CPR)
cefadroxil (CFR)	ceftazidime (CAZ)
cefamandole (FAM)	ceftibuten (CTB)
cefazolin (CFZ)	ceftizoxime (ZOX)
cefdinir (CDR)	ceftriaxone (CRO)
cefditoren (CDN)	cefuroxime (axetil) and
cefepime (FEP)	cefuroxime (sodium) (CXM)

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

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

Antifungal agents. Use the indicated abbreviations for the following antifungal agents.

amphotericin B (AMB)	ketoconazole (KTC)
clotrimazole (CLT)	nystatin (NYT)
flucytosine (5FC)	terbinafine (TRB)
fluconazole (FLC)	voriconazole (VRC)
itraconazole (ITC)	

Antiviral agents. Use the indicated abbreviations for the following antiviral agents.

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

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 10³. Avoid compound prefixes such as mμ or μμ. Use μg/ml or μg/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 “ $\text{nmol}/10 \text{ min}$,” and “ $\mu\text{mol}/\text{g}$ ” is preferable to “ $\text{nmol}/\mu\text{g}$.” 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 “ $\mu\text{mol}/\text{g}/\text{min}$.” 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 article by Olsen (*Infect. Immun.* **71**:6689–6692, 2003).

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

Statistics

Statistical analysis of data is a crucial component of scientific publication. Authors who are unsure of proper statistical analysis should have their manuscripts checked by a qualified statistician.

The following is a list of important items that must be considered before manuscript submission. Deficiencies in any of these areas may delay review and/or publication.

(i) Statistical analyses were performed on all quantitative data regardless of how significant the differences look in the tables or figures.

(ii) Data were appropriately analyzed as parametric (normally distributed) or nonparametric data.

(iii) Parametric and nonparametric data are presented appropriately. Means and standard deviations or standard errors are appropriate means of presenting data analyzed by parametric analyses (i.e., *t* test and analysis of variance [ANOVA]), but only medians and surrounding levels (quartiles, quintiles, and 10th and 90th percentiles, etc.) are appropriate for nonparametric statistics (Mann-Whitney test and Kruskal-Wallis test, etc.). Means have no meaning in nonparametric analyses.

(iv) For any data in which there are more than two comparisons (i.e., between one control and more than one experimental group), an analysis must be done for multigroup comparisons. Such an analysis would usually be an ANOVA for parametric data or a Kruskal-Wallis test for nonparametric data. *t* tests cannot be used when more than two groups are being compared (except as indicated below). Failure to use multigroup tests generates type 1 errors: concluding that two data sets within the overall data set being compared are different when in fact they are not. Exception: some statisticians argue that two-group comparisons can be used on multigroup data if the expected outcomes are appropriately anticipated before the experiment. For example, data generated by individually testing two unrelated factors for their effects on a target with only a single, untreated target as a control could be appropriately analyzed by *t* tests instead of ANOVA.

(v) For all appropriate multigroup comparisons, two *P* values must be generated and provided in the manuscript. The main *P* value applies to the overall data set and indicates that within that data set at least two groups differ from each other. The overall *P* value does not indicate which two groups are

different. The main *P* value and the overall *P* value should be computed by using a *post hoc* test. For ANOVA, these *post hoc* tests are usually Dunnett's test (used to compare multiple experimental groups to a single control), the Fisher protected least significant difference (PLSD) test, the Tukey-Kramer test, and the Games-Howell test. Others may be used. Note that each *post hoc* test has certain underlying assumptions that may not be applicable to the data under analysis. For a Kruskal-Wallis nonparametric ANOVA, the Dunn procedure is appropriate to generate *P* values for two-group comparisons.

(vi) Data presented as endpoints (i.e., LD_{50} and ID_{50} , etc.) contain both the calculated value and a confidence interval with a statistical significance associated with it (95%, 99%, or similar confidence interval), calculated by logit or probit analysis. Simple LD_{50} values, such as Reed-Muench calculations, may not be used alone.

(vii) When samples are taken multiple times from one experimental entity (i.e., multiple serum samples from one animal, gross pathology scores measured for the same animal over time or growth curves, etc.), one cannot use analyses such as *t* tests, ANOVA, or the Mann-Whitney test, etc., because these tests assume that each measure is independent. An entity with a high score on day 1 is more likely to have a high score on day 2 than is an entity with a low score. It is likely that some expert statistical help will be needed for these situations, usually involving regression analysis or survival analysis, etc.

(viii) Statistical significance and biological significance are not the same. There is nothing magical about a *P* value of 0.05. When results from large sample sizes are compared, a *P* value of <0.05 will often be obtained, as *P* value is a function of both sample size and effect size. If sample sizes are large, then more-rigorous (i.e., smaller) *P* values may be desirable. If sample sizes are small, *P* values of >0.05 may still be important. There should be both statistical and biological significance to the results and conclusions in the manuscript.

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

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 $\text{H}_2\text{ }^{35}\text{SO}_4$). 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	UDP-[^{14}C]glucose
L-[methyl- ^{14}C]methionine	<i>E. coli</i> [^{32}P]DNA
[2,3- ^3H]serine	fructose 1,6-[^{32}P]bisphosphate
[α - ^{14}C]lysine	[γ - ^{32}P]ATP