

plastic medical equipment. BMC Infect Dis 2010; 10:92.

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## Reply to Snelling et al

TO THE EDITOR—In their letter, Snelling et al [1] discuss theoretical measurements relating to spore deposition, as opposed to the recovery of spores during air sampling. Crucially, the authors' data do not consider variations in spore size to be secondary to culture conditions, in vitro processing (eg, washing and electron microscopy processing), hydration, and phase of maturation. For example, according to phase-contrast microscopy, *Clostridium difficile* spore populations are often not homogenous; phase-dark spores (hydrated spores before germination) are approximately twice the size of phase-bright spores [2]. More importantly, measurements of spores generated in vitro are of limited significance, because naked spores do not commonly occur in nature—certainly not when generated from fecal matter. In reality, the particles formed will probably be much larger than the size of a single spore and be polydispersed. *C. difficile* spores show a tendency to aggregate, which also makes the aerosolization of single spores unlikely. Thus, it is likely that airborne particles containing *C. difficile* spores will be larger and will settle out rapidly after generation, as is shown in Figure 1 of our article [3]. In fact, the decrease in spore number from 8 to 1 in 15 min we recorded suggests an average deposition rate of ~1 m (assuming this as source height) in 15 min. This equates to a terminal velocity of 1.11 mm/s—10 times the largest velocity suggested by Snelling and colleagues—and a fallout time of <0.25 h, which equates to an average aerodynamic particle size of at least 6.1  $\mu\text{m}$  and an actual diameter of 4.3  $\mu\text{m}$  (using the density value noted below).

The terminal velocity calculations in the table produced by Snelling and colleagues assume a unit density for clostridial spores. In fact, the reported dry density for clostridial spores is 1.42 g/mL [4]. This means that the terminal velocities should be multiplied by 1.42 and the settling time divided by 1.42. For example, the terminal velocity for 1.99- $\mu\text{m}$  particles should be 0.185 mm/s, and the time to deposit 1 m reduced to 1.5 h.

Validation work conducted at the Health Protection Agency's Centre for Emergency Preparedness and Response (A. Bennett, personal communication) determined the extent of deposition on the (12 mm in diameter) inner surfaces of the tubing used during our air sampling. Only 0.14%–15.9% of particles measuring 0.7–17.6  $\mu\text{m}$  (expressed in *Bacillus subtilis* colony-forming units) were trapped on the inner surfaces of the tubing. Thus, such loss had a minimal effect on the airborne *C. difficile* counts we measured in situ [3]. Last, we emphasize that air movements in the clinical setting are extremely difficult to predict. Environmental sampling of sites beyond the reach of routine cleaning clearly show that *C. difficile* is frequently deposited on a variety of surfaces in clinical areas, likely exacerbated by aggregation with organic matter, as discussed above [5].

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## Achieving a Quantitative Understanding of Antiretroviral Drug Efficacy

TO THE EDITOR—We are writing to expand on some issues raised in a recent article by Henrich et al [1] regarding the instantaneous inhibitory potential (IIP), a novel index of antiviral activity developed by Shen et al [2, 3]. The IIP is the log reduction in single-round infection events produced by a drug at clinically relevant concentrations. Because the IIP takes into account the shape (slope) of the dose-response curve, we have argued that it is a more accurate representation of intrinsic antiviral activity than are conventional pharmacodynamic measures, such as the median inhibitory concentration (IC<sub>50</sub>) or inhibitory quotient (the ratio of clinical drug concentration to IC<sub>50</sub>). Importantly, the slope parameter is included in all of the fundamental equations of pharmacology, including the Hill equation [3], the sigmoidal E<sub>max</sub> model [4], and the Chou-Talalay median-effect equation [5]. Thus, a true understanding of the dose-effect relationship cannot be achieved without inclusion of this parameter. It had been largely ignored in studies of antiretroviral drugs until our study showed that the slope parameter varies dramatically and in

a class-specific way for antiretroviral drugs [2, 3]. Indeed, we showed that differences in the slope parameter explain the higher antiviral activity of the nonnucleoside reverse-transcriptase inhibitors (NNRTIs) and protease inhibitors relative to the nucleoside analogue reverse-transcriptase inhibitors. The superior activity of the NNRTIs and protease inhibitors, a well-accepted concept that is incorporated into human immunodeficiency virus (HIV) treatment guidelines [6], is not explained by standard measures, such as  $IC_{50}$  or inhibitory quotient. In this regard, the integrase strand transfer inhibitors (ISTIs) represent a special case, which is discussed below.

Henrich et al [1] examined the relationship between the pharmacologic measures IIP and inhibitory quotient and the outcomes of clinical trials of antiretroviral drugs. Not surprisingly, neither parameter showed a particularly strong correlation with outcome as measured according to the fraction of patients who had an undetectable level of HIV RNA after 48 weeks of treatment. It is important to note that IIP was developed to predict antiviral activity at a given drug concentration, not clinical outcome. As we pointed out in our original article [2, 3], clinical outcome is determined by many factors in addition to antiviral activity, including pharmacokinetics, distribution, toxic effects, adherence, drug interactions, and barriers to resistance. Henrich and colleagues suggest that these factors dominate the slope in determining drug efficacy and cite the superior clinical performance of efavirenz over indinavir as an example. Interestingly, this comparison provides a perfect illustration of how slope influences antiviral activity. Indinavir has a steep slope, which means that small increases in drug concentration produce large increases in antiviral activity. Missing from the analysis by Henrich and colleagues is an appreciation of the fact that a steep slope can also have negative consequences for drug action, because for drugs with a steep slope, small decreases in drug concentration

cause large decreases in activity. For drugs with a steep slope and a short half-life, the IIP falls dramatically during the dosing interval. As we pointed out [2, 3], when this effect is taken into account, efavirenz shows superior activity during the dosing interval, consistent with the clinical data. Thus, we feel that it is important to avoid viewing the role of the slope parameter in an overly simplistic way.

We believe that a true understanding of the efficacy of antiviral drugs requires consideration of 5 factors: (1) the intrinsic antiviral activity of a drug at a given concentration, which, according to fundamental laws of pharmacology, is a function of both  $IC_{50}$  and slope; (2) the change in the concentration of the drug over time (ie, pharmacokinetics); (3) factors such as convenience and toxic effects that determine whether the patient will actually take the drug; (4) interactions with other drugs in the regimen; and (5) genetic barriers to resistance in cases in which suppression is suboptimal. The superior performance of protease inhibitors such as darunavir is largely a result of the first factor [2, 3], whereas for the ISTIs, the fourth factor is of particular importance (B. Jilek, M. Sampah, L. Shen, and R. Siliciano, unpublished data, 2010). Our hope is that a quantitative analysis of all 5 factors will eventually allow a more rational choice of treatment regimens, particularly in patients with resistance.

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## Response to Shen and Siliciano

In their letter regarding our article comparing the instantaneous inhibitory potential (IIP) and the inhibitory quotient as predictors of antiretroviral efficacy [1], Shen and Siliciano [2] propose that understanding the efficacy of antiretroviral drugs requires consideration of 5 factors: intrinsic antiviral activity, pharmacokinetics, convenience and tolerability, drug-drug interactions, and the genetic barrier to resistance. We agree. In our view, the examples of efavirenz and indinavir illustrate the point that pharmacokinetics may trump intrinsic activity in determining relative efficacy. Comparative trials involving treatment-naïve patients have generally not revealed superior efficacy when drugs with higher IIPs have been tested against drugs with lower IIPs (ie, efavirenz vs nevirapine [3], efavirenz vs atazanavir [4], darunavir-ritonavir vs lopinavir-ritonavir [5], lopinavir-ritonavir vs fosamprenavir-ritonavir [6], and saquinavir-ritonavir vs lopinavir-ritonavir [7]). It will be interesting to see whether the higher intrinsic activity and IIP of darunavir-ritonavir (IIP, 8.46 [2]) translates into superior efficacy,