India could be exposed to different risk factors from populations living in urban areas.[2] For example, maternal malnutrition might have a major effect on the risk of vascular disease. About three-quarters of the world's malnourished live in rural areas[3] and as many as 48% of pregnancies in rural India are complicated by intrauterine growth retardation.[4] The strong inverse relation between low birthweight and adult blood pressure could therefore be a major factor in consequent vascular disease in this setting.[5] Moreover, hypertension has its own risk factors, and those present in disadvantaged communities might differ from those present in more affluent regions.

We thus propose an important caveat to the interpretation of the INTERSTROKE data; the ten identified risk factors, which account for 90% of stroke risk in the INTERSTROKE population, might not apply to the same extent, or indeed some might not be relevant, to people living in rural disadvantaged settings. Such populations still remain to be studied.

We declare that we have no conflicts of interest.

*Amanda G Thrift, Velandai Srikanth, Roger G Evans
amanda.thrift@bakeridi.edu.au

Baker IDI Heart and Diabetes Institute, Melbourne, VIC 3004, Australia (AGT); and Departments of Epidemiology & Preventive Medicine (AGT), Medicine (VS), and Physiology (RGE), Monash University, Melbourne, VIC, Australia

1 O'Donnell MJ, Xavier D, Liu L, et al. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. *Lancet* 2010; **376:** 112–23.

2 Thrift AG, Srikanth VK, Fitzgerald SM, et al. The potential roles of high salt intake and maternal malnutrition in development of hypertension in disadvantaged populations. *Clin Exp Pharmacol Physiol* 2010; **37:** e78–90.

3 Human Development Report 2007/2008. Fighting climate change: human solidarity in a divided world. New York: UNDP, 2007. http://hdr.undp.org/en/reports/global/hdr2007-8/ (accessed Oct 16, 2010).

4 de Onis M, Blossner M, Villar J. Levels and patterns of intrauterine growth retardation in developing countries. *Eur J Clin Nutr* 1998; **52** (suppl 1): S5–15.

5 Davies AA, Smith GD, May MT, Ben-Shlomo Y. Association between birth weight and blood pressure is robust, amplifies with age, and may be underestimated. *Hypertension* 2006; **48:** 431–36.

Authors' reply

Amanda Thrift and colleagues highlight an important potential limitation of phase 1: that populations from rural and disadvantaged settings in low-income countries might not have been adequately represented.

To explore this issue further, we looked at: (1) whether participants with stroke lived in a rural, suburban, or urban setting (from questions designed to measure exposure to outdoor pollution); and (2) years of formal education completed (a surrogate for socioeconomic status in childhood). Overall, almost half reported that they lived in a rural (19%) or rural/suburban (28%) environment and 16% of the populations did not receive any formal education. Phase 2 will include a much larger sample from more diverse regions, and will seek to expand the proportion of rural-based centres in low-income and middle-income countries.

Thrift and colleagues also highlight the need to measure the societal determinants of traditional vascular risk factors for stroke, such as hypertension, which can vary by region and within regions, and by urban versus rural location. To address this crucial issue, our group is doing the Population Urban Rural Epidemiology (PURE) study,[1] which has enrolled 151 000 individuals residing in about 600 communities (about half urban and half rural) in 17 high-income, middle-income, and low-income countries, and which will look at the underlying determinants of cardiovascular disease and traditional risk factors.

We declare that we have no conflicts of interest

*Martin O'Donnell, Purnima Rao-Melacini, Salim Yusuf
odonnm@mcmaster.ca

Henderson Research Center, Hamilton, Ontario L8V 1C3, Canada

1 Teo K, Chow CK, Vaz M, Rangarajan S, Yusuf S; PURE Investigators Writing Group. The Prospective Urban Rural Epidemiology (PURE) study: examining the impact of societal influences on chronic non-communicable diseases in low-, middle-, and high-income countries. *Am Heart J* 2009; **158:** 1–7.

Biomarkers and diagnostics for tuberculosis

Science Photo Library

In panel 2 of their Article on biomarkers and diagnostics for tuberculosis (May 29, p 1920),[1] Robert Wallis and colleagues highlight that the time to detection of multidrug-resistant tuberculosis with the non-commercial nitrate reductase assay (NRA) in indirect application would not be faster than conventional drug susceptibility testing with solid culture. This is surely a mistake.

As summarised in panel 3 of the same Article and in another publication by some of these authors, the NRA is faster than the gold-standard method on solid medium.[2] It has been shown that the result time with NRA is similar to that of the liquid culture systems, and this characteristic was mentioned in the report of the ninth meeting of WHO's Strategic and Technical Advisory Group on Tuberculosis.[3]

Wallis and colleagues also comment that non-commercial assays are not standardised and need extensive training, optimisation, and quality assurance before clinical use. Meticulous training and quality assurance are also required for commercial assays. Procedures to identify multidrug-resistant tuberculosis accurately by use of non-commercial assays have been consistently and extensively detailed.[4] In this regard, the only concern raised in the Strategic and Technical Advisory Group report refers to microscopic-observation drug susceptibility (MODS), which was allegedly developed to be used in

laboratories that meet the biosafety level required for culture. Unless researchers adequately address the speciation of *Mycobacterium tuberculosis*, MODS should be implemented only in laboratories with appropriate biosafety measures for handling positive cultures of the tubercle bacilli in liquid media.

We declare that we have no conflicts of interest.

*Anandi Martin, Lucia Barrera, Juan Carlos Palomino
amartin@itg.be

Institute of Tropical Medicine, 2000 Antwerp, Belgium (AM, JCP); and Instituto Nacional de Enfermedades Infecciosas ANLIS Carlos Malbran, Buenos Aires, Argentina (LB)

1 Wallis RS, Pai M, Menzies D, et al. Biomarkers and diagnostics or tuberculosis: progress, needs, and translation into practice. *Lancet* 2010; **375:** 1920–37.
2 Pai M, Minion J, Sohn H, Zwerling A, Perkins MD. Novel and improved technologies for tuberculosis diagnosis: progress and challenges. *Clin Chest Med* 2009; **30:** 701–16.
3 WHO. Report of the 9th meeting of the Strategic and Technical Advisory Group on Tuberculosis (STAG-TB). Geneva: World Health Organization, 2009. http://www.who.int/tb/advisory_bodies/stag/en/index.html (accessed March 24, 2010).
4 Martin A, Panaiotov S, Portaels F, Hoffner S, Palomino JC, Angeby K. The nitrate reductase assay for the rapid detection of multidrug resistant *M tuberculosis*: a systematic review and meta-analysis. *J Antimicrob Chemother* 2008; **62:** 56–65.

Authors' reply

The first point raised in Anandi Martin and colleagues' letter relates to the speed of the nitrate reductase assay (NRA) method for detecting drug resistance in tuberculosis, and questions the accuracy of the text in panel 2 of our paper. Panel 2 was a summary of WHO policies and statements on tuberculosis diagnostics. According to the WHO policy statement on non-commercial culture and drug-susceptibility testing methods for screening of patients at risk of multidrug-resistant tuberculosis,[1] "NRA can be used as a direct test on smear-positive sputum specimens or as an indirect test on *M tuberculosis* isolates grown from conventional solid culture. Indirect testing using NRA is therefore not faster than conventional phenotypic DST using solid media."

The second issue raised by Martin and colleagues was whether there were special considerations for training, standardisation, optimisation, and quality assurance for non-commercial compared with commercial products. Undoubtedly there are. Although adequate training is crucial irrespective of what type of diagnostic is being used, well manufactured commercial assays (under ISO:13485 or similar standard) will have been standardised, optimised, and quality controlled by the manufacturer. The quality control process would include lot release testing, which is widely accepted as replacing the need for routine local media quality control by professional associations in developed countries.[2]

The issue with non-commercial culture methods is not whether they *can* perform well, but how they will perform well if use b ecomes widely disseminated, including into laboratories with limited human resource capacity. This potential for variability in performance of non-commercial assays could be compounded by the individual selection of assay components (antibiotics, plastics, media preparation reagents) for procurement by laboratories. These concerns apply not only to NRA, but to other methods such as colorimetric redox indicator (CRI), microscopic-observation drug susceptibility (MODS), and thin-layer agar.[3] As regards biosafety, the WHO policy recommends that NRA, CRI, and MODS are suitable for use at reference laboratory level, under strict laboratory protocols.[1]

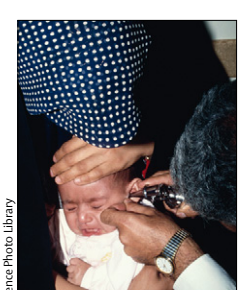
In summary, although they might be substantially less expensive, one disadvantage of non-commercial culture systems highlighted by the limited WHO policy recommendations is that they transfer all the requirements for standardisation and quality assurance onto local laboratories, which are unfortunately often poorly equipped to do these tasks. Indeed, the WHO policy emphasises that "non-commercial methods are prone to errors related to lack of standardization and due to local variations in methodology."[1] This point serves to further emphasise the crucial need for strengthening laboratory capacity in countries with a high burden of tuberculosis.[4]

MPe is the Chief Scientific Officer of Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland, a non-profit agency that works with several industry partners in developing and evaluating new diagnostics for neglected infectious diseases. MPa serves as an external consultant for FIND; serves as a co-chair of the Stop TB Partnership's New Diagnostics Working Group; and serves as chair of the Task Force of the Stop TB Research Movement.

*Mark Perkins, Madhukar Pai
mark.perkins@finddiagnostics.org

Foundation for Innovative New Diagnostics (FIND), 1202 Geneva, Switzerland (MPe); and McGill University, Montreal, Canada (MPa)

1 WHO. Non-commercial culture and drug-susceptibility testing methods for screening of patients at risk of multi-drug resistant tuberculosis: policy statement (July, 2010). http://www.who.int/tb/dots/laboratory/whopolicy_noncommercialculture_and_dstmethods_july10.pdf (accessed Sept 2, 2010).
2 Australian Society for Microbiology. Guidelines for assuring quality of medical microbiological culture media. http://www.theasm.org.au/uploads/pdf/Guidelines%20for%20Assuring%20Quality%20of%20Medical%20Microbiological%20Media.pdf (accessed Oct 21, 2010).
3 Minion J, Leung E, Menzies D, Pai M. Microscopic-observation drug susceptibility and thin layer agar assays for the detection of drug resistant tuberculosis: a systematic review and meta-analysis. *Lancet Infect Dis* 2010; **10:** 688–98.
4 Nkengasong JN, Nsubuga P, Nwanyanwu O, et al. Laboratory systems and services are critical in global health: time to end the neglect? *Am J Clin Pathol* 2010; **134:** 368–73.



Science Photo Library

Family physicians in Iran: success despite challenges

Although Keivan Shalileh and Abolfazl Mahdanian correctly mention the problems of being a family physician in Iran (Aug 14, p 515),[1] the current achievements of the Family Physician Project should not be overlooked. The number of physicians in rural areas (those with <20 000 inhabitants) has increased from less than 2000 in 2005 to more than 6000 in 2006, just 1 year after implementation of this programme,