

Rapid Influenza Testing in an Austere Setting, Mongolia

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In 2015–2017, we helped rural Mongolian clinicians with poor infrastructure adopt rapid influenza diagnostic tests (RIDTs). In their hands, the Quidel Sofia Influenza A Test was both sensitive (75%) and specific (100%). If made widely available, such RIDTs would have the potential to markedly reduce influenza morbidity and mortality in Mongolia.

Keywords. influenza; Mongolia; RIDT.

Mongolia, since it won independence from the former Soviet Union in 1980, has had many challenges in modernizing its clinical services. The country is relatively large and sparsely populated, with extreme shifts in weather (+33°C to –30°C) [1], unstable electrical supply, questionable potable water sources, primitive roads, and recent economic distress. While Mongolia has established an admirable National Influenza Center (NIC), which has increased its national surveillance to dozens of sites since its establishment in 2004–2005 [2], these challenges often render NIC influenza data more important to public health officials than to individual patients. Yet, the need for such clinical diagnostics in rural Mongolia is great as influenza-like illnesses are relatively common during winter months [3] and large outbreaks [4] have occurred. In this study, we sought to evaluate rapid influenza diagnostic test (RIDT) use in rural Mongolia, where influenza diagnostics are seldom used and empiric therapy is the standard of care.

METHODS

During September 2015, more than 10 professionals from the National Center for Zoonotic Disease (NCZD) branches in

Arkhangai, Uvurkhangai, and Umnugobi aimags (rural provinces) and Ulaanbaatar were trained at the NCZD headquarters in Ulaanbaatar in enrolling patients through informed consent, collecting nasal pharyngeal specimens, and performing the Quidel Sofia Influenza A+B assays. These clinical branches provide public health support in rural areas but lack any respiratory virus diagnostics. Further training of the rural professionals and aimag clinicians was performed several months later when the study team first visited each aimag.

Ethical Approval and Sampling

This study was approved by Duke University and the Mongolian Ministry of Health Institutional Review Boards.

Clinical Definition

We sought to evaluate RIDT assay use among patients with severe acute respiratory illness (SARI). A SARI case was defined as a hospitalized patient who presented with coughing, a fever >38°C, and an onset of symptoms within the last 10 days. Local hospital staff confirmed that the patient met the case definition for SARI, gained informed consent, completed a SARI Patient Enrollment Form, and asked the health care provider to complete a Physician Rapid Test Survey.

Sample Collection

Two nasopharyngeal (NP) swabs were collected from each hospitalized patient who met the case definition for SARI. Each nonflocculated swab was placed in viral transport media (3 mL of UTM; Universal Transport Medium, Copan Diagnostics, Italy). One NP swab specimen was studied in the field with a rapid test. The second NP swab specimen was preserved at –4°C until transported (within ~72 hours of collection) to the National Center of Zoonotic Diseases (NCZD) or the Institute of Veterinary Medicine (IVM), both in Ulaanbaatar. Upon arrival, these specimens were preserved at –80°C until studied with confirmatory real-time reverse transcription polymerase chain reaction (qRT-PCR) assays.

Rapid Testing

Rapid testing was performed in the field using Quidel's Sofia test (Quidel Corporation, San Diego, CA), run from December 2016 to January 2017.

Molecular Assays

At the NCZD or IVM, total nucleic acid was extracted from 140 µL of NP swab samples using the Qiagen extraction system: QIAamp Viral RNA Mini Kit (Qiagen Inc., Valencia, CA) following a mini-spin protocol. A World Health Organization qRT-PCR [5] procedure was used to screen NP respiratory specimens for influenza A and B virus. Influenza A–positive specimens were further examined with a qRT-PCR H3–specific assay.

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A positive qRT-PCR assay was defined as having a Ct value ≤ 38 , and a suspect-positive assay was defined as having a Ct value between >38 and <40 . Suspect-positive assays were repeated and considered positive only if the repeat Ct value was ≤ 38 . Positive and negative controls were used in each molecular assay run.

Statistical Analysis

Descriptive statistics were run using Microsoft Excel 2016 or Epi Info, version 7.2 (Centers for Disease Control and Prevention, 2017). Sensitivity and specificity analyses were performed with MedCalc Software [6].

RESULTS

During December 2015 to March 2017, 75 patients with SARI were enrolled in a convenience sample through informed consent: Arkhangai aimag ($n = 17$), Uvurkhangai aimag ($n = 33$), and Umnugobi aimag ($n = 25$). Patients ranged in age from 5 months to 70 years (mean, 10.6 years) and were 56% male ($n = 42$). Overall, 12 patients' specimens were positive by qRT-PCR assays for influenza A and 1 for influenza B (Table 1). However, not all specimens were tested with the Sofia system as late in the study the kits expired. Fifty-nine of 75 patients had their NP swabs tested with both the Sofia and qRT-PCR assays. Eight were positive for influenza A, and 1 was positive for influenza B. The sensitivity and specificity of the Sofia system for influenza A were similar to reports from their use in developed countries [7] (influenza A sensitivity, 75%; 95% CI, 34.9%–96.8%; specificity, 100%; 95% CI, 93%–100%).

Rural clinicians commented that such rapid diagnostics were greatly needed in their small communities, where they are often forced to treat patients empirically. Rapid tests were valued in their promise to shorten the time needed to prescribe targeted treatments.

DISCUSSION

In this study, we sought to evaluate the use of RIDTs in rural Mongolia, where laboratory virus detection was essentially nonexistent. Rural clinicians told us that while they could send clinical specimens to the capital of Ulaanbaatar for molecular testing, the delays in gaining test results (sometimes 1 to 2 weeks), limit the clinical value of such testing. Without specific

influenza diagnostics, few clinicians would be willing to prescribe expensive antivirals, even if they were available and could reduce morbidity and save lives.

Our results documented a relatively high sensitivity and specificity for the RIDT use we supported in rural Mongolia, demonstrating the feasibility of training rural Mongolian clinicians and laboratory staff in RIDT use. Adopting RIDTs seems to these authors as a practical, morbidity and mortality-reducing step forward in modernizing Mongolia's clinical care in rural settings. The key will be the cost and availability of RIDTs as Mongolia has little capacity for developing its own diagnostics.

However, it now seems likely that Mongolia's access to inexpensive RIDTs could be further constrained. The US Food and Drug Administration (FDA) has recently called for diagnostic companies to meet new higher sensitivity and specificity benchmarks by January 2018 to continue to market RIDTs in the United States [8]. From observations at a recent international clinical virology meeting, this seems to be nudging US diagnostic companies with previously approved Clinical Laboratory Improvement Amendments (CLIA)-waived, later-flow antigen detection assays to move toward developing minimally complex, tabletop, cartridge-based molecular assays and abandoning their older assays. While this movement is a very good thing for patients and health care providers who have the resources to support these new, more accurate but higher-cost assays, in developing countries the new molecular assays may be cost-prohibitive at a time when RIDT use is just now becoming accepted in clinical care.

This study had a number of limitations. We only studied 3 rural areas, and as much of Mongolia is similarly rural, these data should not be construed as nationally representative. Despite intensive training and written standard operating procedures, delays in rapid testing use may have occurred for at least some of the 75 NP swab specimens. Such delays, despite specimen refrigeration, may have resulted in reduced RIDT sensitivity. Similarly, while we assessed compliance through multiple training sessions and on-site observations, some NP specimen collections may not have been optimally collected. Even so, the RIDTs used in rural Mongolia had sensitivity and specificity statistics similar to statistics in reports from developed countries, supporting their use in these rather austere settings.

Table 1. Laboratory Assay Results Among 75 Study Subjects Identified as Meeting Case Definition for Severe Acute Respiratory Infections: Quidel Sofia Influenza A+B Test and World Health Organization real-time RT-PCR Influenza A and B Assays

Aimag	SARI Samples	Sofia-Positive Influenza A ^a	Sofia-Positive Influenza B ^a	Real-time RT-PCR-Positive Influenza A, %	Real-time RT-PCR-Positive Influenza B, %
Arkhangai	17	1	1	4 (23.5)	0 (0.0)
Uvurkhangai	33	1	0	3 (9.1)	0 (0.0)
Umnugobi	25	4	1	5 (20.0)	1 (4.0)
Total	75	6	2	12 (16.0) ^b	1 (1.3)

^aOnly 59 of the 75 patient specimens were tested with the Quidel Sofia Influenza A+B. All specimens were tested with the World Health Organization real-time RT-PCR influenza A and B assays.

^bOnly 8 of these 12 positive specimens were examined with both the Quidel Sofia Influenza A+B and the World Health Organization real-time RT-PCR influenza A assay.

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