Logistic aspects of a deployable molecular microbiology laboratory

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Abstract

A molecular diagnostic laboratory was deployed overseas in response to a suspected emerging infectious disease in Sri Lanka in early 2008. The equipment and procedures used are established technology, well within the capability of a large hospital laboratory. However, the main obstacles to operating these systems in a resource-limited, tropical environment are better understood with reference to the logistic envelope of demand, distance and duration. Careful attention to basic logistic principles contributed to the success of this laboratory deployment and established a foundation for future technical enhancements. The lessons learned have further implications for pathology support operations and field hospital planning.

Introduction

Infectious diseases cling to military campaigns with a predictable inevitability. It has been clear that infection contributes a major proportion of diseases and non-battle injuries ever since the introduction of formal epidemiological analysis to military medicine. As recently as the initial NATO deployment into Afghanistan, allied forces estimated that infections contributed around 12% loss of combat availability[1]. Despite recent technical advances, it has proved difficult to insert advanced diagnostic capability into a theatre of operations and sustain clinical microbiology support for the duration of deployment. The reasons are many. In general they reflect the mismatch between a heavy burden of endemic and epidemic infectious disease on the one hand, and the diagnostic tools available to allied defence health units on the other. A specific reason for this is the increasing reliance of civilian public health on highly centralized laboratories where molecular diagnostic tests can be performed with high capacity, high throughput equipment and specialized staff. Very little diagnostic molecular microbiology is performed outside the larger population centres. This challenge has been particularly evident in the remote communities of tropical northern Australia. An operational concept was developed for a deployable biological threat assessment capability during a series of studies in the West Kimberley[2]. The portable molecular diagnostic tools used became the platform for an emerging infectious disease response in Sri Lanka. The scientific and technical aspects of that deployment were an application of existing technology[3]. A far bigger challenge was resolution of the logistic obstacles to an overseas deployment. Previous attempts to operate a limited version of this platform in Brazil were unsuccessful[4]. The purpose of this paper is to describe the logistic solutions that were developed and explore the reasons for previous failures in order to develop a concept of operations for field-deployable molecular microbiology.

Methods and logistic process

Existing laboratory infrastructure. Outside Colombo, the clinical microbiology laboratories in Sri Lanka lack advanced diagnostic capability. In Peradeniya, there were none of the molecular microbiology facilities Australian teaching hospitals expect to operate. This lack of molecular and other advanced microbiological equipment meant that the field deployable laboratory had to be completely self-sufficient apart from a single conventional incubator.

Method development.

The methods used to set up from scratch were developed around equipment believed to be sufficiently robust to operate in an austere laboratory environment after transfer by international freight, including surface shipment at ambient temperature and unpacking. Real-time thermal cyclers were avoided, molecular methods pared down to their barest minimum, and laboratory procedures tested to find their weak points. Procedures were rehearsed with the equipment and reagents planned for use in Sri Lanka. Four key methods were chosen: preliminary culture of organisms using selective bacteriological agar,

DNA extraction, DNA amplification and amplicon resolution. Scientific procedures were as previously described[3]. In brief these were as follows: clinical bacterial isolates were obtained from two hospitals in Sri Lanka and subcultured on the bacteriological media normally used for preliminary isolation from clinical samples. Environmental samples were cultured on selective agar made from reagents brought in with the deployable laboratory according to a previously published recipe[4]. Bacterial DNA was extracted from these bacterial cultures as described [3]. These solutions were used to provide template material for the polymerase chain reaction (PCR). Instead of running the amplified DNA product through gels in the conventional manner, PCR products were run in a disposable microfluidic lab chip. The results were analysed using the bioanalyser software (Expert 2100, Agilent Technologies, Waldbronn, DE).

Logistics.

Preparations were made to operate in a restricted range of molecular microbiology tests at an extended distance from the sending base without resupply, repair or trouble-shooting support for a period of up to one week. Survivability was planned into the operation by duplication of critical consumables, small equipment items, and alternate onward shipping arrangements. Pre-departure testing to failure and repeated mission rehearsal enabled refinement of the protocols and identification of critical reagent requirements. This assisted plans for international shipment of equipment, frozen, refrigerated and ambient temperature reagents and the corresponding travel arrangements for laboratory staff. The list of materials required was converted into a laboratory manifest, and then into a series of contents lists for each freight package. These were governed by temperature requirement, weight, quarantine clearance, anticipated import duty and potential for substitution. The logistic nodes and links are shown in Figure 1.

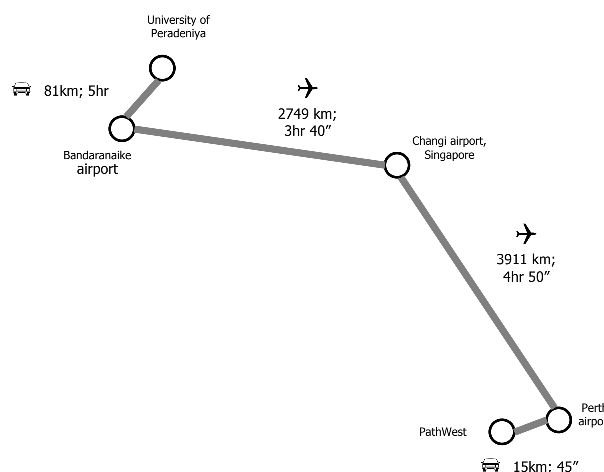

Figure 1: Logistics nodes and links

Table 1: Shipping arrangements for deployable laboratory equipment, reagents and other supplies

Contents	Temperature	Agency	Customs	Cost
Equipment: Bioanalyser Thermal cycler Heating block Vortex mixers Automatic pipettes Other non-consumables	ambient	Freight forwarder, Chamber of Commerce, & return freight forwarder	Carnet (exemption from duties)	$528 (Carnet) $1108 (return)
Laboratory consumables, Non-perishables including agar concentrate	ambient	Courier	Standard, formalities by courier	$2015.68 Inclusive of all 3 couriered packages
Cold sensitive laboratory Consumables including lab chip reagents	1-4°C	Courier, as above	Standard, formalities by courier	Included in above sum
Frozen perishable reagents including PCR reagent components	-20°C	Courier, as above	Standard, formalities by courier	Included in above sum

Notes

The equipment trunk was dispatched as soon as the Carnet was ready, one week before the last possible date of arrival in Sri Lanka in order to coordinate with personnel arrival at the international airport. Laboratory reagents and consumables were dispatched just prior to that and couriered through to the final destination to arrive during working hours and therefore reduce the risk of interruption of the cold chain.

Results

Plans to send a scientist and a pathologist from Perth had to be revised due to the deteriorating security situation and the corresponding Australian government travel advisory. The pathologist travelled to Sri Lanka without an accompanying scientist. His preparation had therefore to include the entire proposed PCR method, the ability to train others in its operation and troubleshooting i.e. complete self-sufficiency. In view of this, we chose a single DNA target and a corresponding PCR protocol. This simplified the protocol development and reagents list. However, the reagents, consumables and equipment needed still required a total of four boxes – three at different temperatures, and a trunk for the laboratory equipment (See Table 1 on page 7).

A customs certificate or "carnet" was obtained to help reduce customs duty payments. This was deemed necessary even though the equipment was due for return to Perth and the reagents were for non-commercial use. Air freight costs were reduced by packing the equipment in an aluminium-reinforced trunk, to reduce the risk of damage during transit. On arrival we were able to confirm that the electronic equipment was intact and functional, but one non-essential consumable (bovine serum albumin; BSA) had been removed and two small equipment items damaged during progress through Customs (lab chip priming station retainer clip, and minishaker restrainer bar). No explanation was given, but there was sufficient backup in the reagent manifest to proceed as planned. Protocol rehearsal prior to departure enabled improvisation to overcome the minor equipment damage. Selective agar was prepared from dried ingredients on arrival. Field surveys were conducted on the day of arrival and the following day, allowing early inoculation of environmental samples from rice terraces and a rubber plantation.

The deployable molecular diagnostic laboratory was successfully set up in Peradeniya in January 2008 and ran for one working week, until the exhaustion of all molecular reagents. The three-step molecular protocol (extraction, amplification and resolution) was run for the first time on day two, using clinical isolates assembled in Peradeniya. One of these isolates was used to obtain positive controls. Though positive and negative results were obtained from the first chip, a single false positive control result indicated possible carry-over during chip inoculation or shaking. This latter possibility was considered most likely due to a missing component of the mini-shaker which had disappeared during transit. Subsequent chips were more carefully secured with masking tape, and there were no further false positive results. Six chips were run without interruption. The seventh was affected by a power outage during loading of the marker ladder and was therefore abandoned. Results provided preliminary confirmation of melioidosis in two patients. Direct testing of soil suspension supernatants produced several positives from a rice terrace and a rubber plantation. Further primary isolation from soil preparations was not possible due to time constraints. Three local staff were trained to use the standard procedures from start to finish in a see one, do one, train one format.

Discussion

This was the first time a molecular method has been used for the detection of melioidosis in Sri Lanka. We operated a small molecular diagnostic laboratory overseas, demonstrating its feasibility and clinical value[3]. Six consecutive lab chips were run successfully. Local staff were given hands-on training in these methods and demonstrated proficiency in running all stages in a series of standard procedures. A larger group of staff were able to see how easily these procedures could be performed. The loss of an experienced scientist from the deploying team restricted the range of tests that could be operated, and emphasised the value of having a range of laboratory skills to call upon. Reliance on one member of the visiting staff reduced the survivability of the deploying laboratory. On the other hand, this also gave skills transfer a greater urgency. A larger team would have allowed greater flexibility of operation, improved supervision of local staff as they trained with the equipment and would have enabled concurrent operations with greater sample processing efficiency and even shorter time to completion. Interestingly, it is now possible to see the failure to produce PCR results during a previous expedition to north eastern Brazil in terms of dependency on unfamiliar local equipment, the more cumbersome gel–based resolution of PCR products and communication in a second language. The success of the present deployment underscores the importance of operational self-sufficiency and mission rehearsal using the precise reagents and equipment intended for the specific expedition.

Future capacity-building expeditions will be required to expand the limited repertoire developed during this first deployment, which could be regarded as a reconnaissance-in-depth. As such it explored a series of logistic obstacles to an international laboratory response to an emerging infection. Logistics is the time-sensitive placement of mission-critical resources. The logistic challenges faced included the need for responsiveness, simplicity, economy, flexibility, balance, foresight, sustainability and survivability. Our use of a robust system capable of operating in austere laboratory conditions after lengthy transit was vindicated by obtaining readable results on our first complete run, despite the loss of small components

and a reagent en route. Careful attention to controls, particularly a positive reading from a negative control and repetition of the first chip run enabled correction and adjustment to the procedure and a series of successful runs from that point. We doubt that a real-time thermal cycler would have produced satisfactory results without a lengthy installation and validation period in these conditions. Rugged, real-time thermal cyclers are available to military establishments or possibly as prototype devices. These hold some promise for the future, but were not yet available to us for use in the Sri Lanka expedition. There is a well-recognised mismatch between the main emerging infectious disease hotspots and the best laboratory response capacity[6]. We were able to adapt commercially available equipment to address some of this technology discrepancy. However removal of the bovine serum albumin concentrate from the reagent shipment during transit prevented us from dispensing additional PCR mastermix and restricted us to a maximum of 96 assays. There was sufficient pre-dispensed mastermix in the original set of imported reagents to run the assay for four consecutive days.

The lessons learned during the Sri Lanka expedition can be applied to military health operations. Very few such deployments are as short as one week or focus on a single infectious disease entity. However, the commoner and more serious infections can often be predicted in many theatres of operations prior to deployment. The laboratory can therefore build up a stock of specific reagents suited to the appropriate PCR methods. Just as a field hospital has a culminating point based on a number of procedures or beds available, the laboratory has a maximum test capacity. An alternative option for operation over extended periods in tropical conditions is shipment of laboratory reagents in freeze-dried form; a service currently available from some of the larger reagent suppliers. In epidemic conditions it may be possible to triage the use of scarce reagents while still providing health commanders high quality, specific diagnostic support. Our experience in Sri Lanka suggests that capacity could be substantially increased for surge response by use of a second thermal cycler and bioanalyser set. This would increase test flexibility, guard against equipment failure and more than double throughput. The portability of the equipment platforms we used enabled us to operate independently of the sending base for a week. Longer term laboratory operations would require resupply, a two-way cold chain, confirmatory test back up and quality assurance support. Additionally, a compact UPS with a reserve capacity of at least 15 minutes would guard against temporary power outages or brownouts as were experienced in this deployment. At present, reliance on support from the sending base is often used to provide basic diagnostic capability. The initial diagnostic test results become irrelevant by the time they reach the treating physician, even if they reliable. We recognise that an unusual mixture of logistic and laboratory skill was required to deploy the molecular laboratory, but a measure of its success was due to the deliberate choice of simple and streamlined procedures, robust equipment and a comprehensive set of controls. The main criticism of the procedures we deployed and operated in Sri Lanka was their lack of flexibility. The test repertoire was tightly restricted and very focused. Flexibility was provided by the training and experience of the operators. Significant expansion of the test repertoire will be needed for military laboratory deployments. The priority diagnostic test capability should be based on the small range of infectious diseases commonly associated with humanitarian disasters, which are also common in locations where the Defence Forces deploy to support United Nations operations. The added complexity of operating a range of diagnostic options will place a greater training and quality control burden on military laboratory staff since deployed laboratories operate beyond the reach of conventional civilian laboratory accreditation processes. Close cooperation with teaching centre and reference laboratories in the national sending base will be required to ensure consistency of molecular results delivered in a deployed setting.

In conclusion, a molecular diagnostic laboratory was successfully assembled, prepared and deployed in a demanding, resource-limited overseas environment. The procedures used are easily adaptable to a variety of monoplex and multiplex diagnostic PCR tasks. The lead time of one month's preparation for deployment was dictated by a combination of training needs, freight times and customs formalities. The extended distance over which we operated meant that the supply chain was stretched over two long-haul flights, an overnight stopover and a lengthy road journey. Further work needs to be done educating Customs and other officials in internationally agreed standards for shipment of diagnostic reagents and support equipment. Customs and quarantine formalities remain a critical failure point in the laboratory deployment process.

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