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Laboratory Capacity for Surveillance of Infectious Diseases in Gujarat: Quantity, Quality, Effects and Way Forward

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Abstract

Background: India carries one of the largest burdens of infectious diseases in the world. To estimate this, laboratory confirmation is vital. We estimated the lab capacity and effectiveness in the state of Gujarat for Enteric Fever, Infectious Hepatitis, and Dengue. **Methods:** We estimated the number of labs in the state through telephonic surveys and physical screening of a representative sample of labs. We created four levels of tests, Level-0 being no test and 3 being the best confirmatory test available in the state. For the profusion of rapid diagnostic test kits (RDTKs), we constructed Effective Diagnostic Scores (EDS) calculated from their sensitivity and specificity at disease prevalences specified in the literature. Tests with EDS > 0.51 were level-2 tests, and EDS < 0.50, level-1 tests. **Results:** Our analysis showed that there are 4293 labs in the state (1765 public and 2528 private), 7/100,000 population. However, only 2878 labs contributed to a total pooled Effective Lab Diagnostic Score (ELDS) of 6776 in the state. Strikingly, 94% of the lab effectiveness lay in RDTs (level-2 and 1 tests) which are essentially screening tests. Ninety-six percent of the overall lab effectiveness of Gujarat existed in private and only 4% in public labs. Contrarily, the level-3 confirmatory testing effectiveness, through ELISA and culture constituted only 4% of private and 36% of public lab effectiveness. More than half of the private lab effectiveness was located in eight Tier 1 cities. Level-3 confirmatory testing effectiveness was present only in Tier 1 and 2 towns. Hepatitis B testing contributed 34% of the total ELDS, followed by Dengue (30%), Enteric Fever (26%) and Hepatitis A and E (10%). **Conclusion:** Our study has established that the capacity and effectiveness of the lab network in Gujarat lie predominantly in RDTKs. We

need to adapt our systems to capture this data in a manner that will allow us to monitor the burdens of these diseases.

Keywords

Rapid Diagnostic Test Kits (RDTKs), Enteric Fever, Hepatitis, Dengue, Lab Diagnostic Effectiveness, Integrated Diseases Surveillance Program (IDSP), India, Gujarat

1. Introduction

India carries one of the largest burdens of infectious diseases in the world. A high density of vectors, vertebrate hosts, and the local ecology actively perpetuate this burden. A robust and effective infectious disease surveillance system, which channels the collection of disease data and its laboratory confirmation, is imperative to contain the amplification cascade [1] [2]. The advantages of laboratories in infectious disease surveillance are manifold. Laboratory investigations can augment the temporal and spatial analysis of surveillance data, leading to befitting response strategies towards disease outbreaks and their changing patterns [3] [4]. Strategic incorporation of both technologically advanced tests as well as rapid diagnostic methods into the lab network can help bridge the gap between detection, surveillance and response to outbreaks [5] [6] [7].

India has had vertical programs and infrastructure (including dedicated laboratories) for diseases such as malaria [8] [9] [10], TB [11], polio [12] [13] and HIV [14] since the last few decades. However, there has not been a determined response to diseases such as enteric fever, hepatitis, dengue, cholera, chikungunya, measles, and diphtheria. In order to be able to control these diseases, diagnosing them accurately is an essential first step. These diagnoses are done by the regular public and private laboratories in the country.

The agency entrusted with the function of Infectious Diseases Surveillance in the state of Gujarat in India is the Integrated Diseases Surveillance Program (IDSP). This program generates weekly surveillance reports for a set of high priority diseases in nine states in the country since 2005 [15]. This is done through the weekly submission of Syndromic (S), Probable/Presumptive (P) and Lab (L) forms by reporting units at district offices, which are then collated and analyzed at the state level. The IDSP lays out case definitions to report Probable and Confirmed cases of 22 infectious diseases [16]. To report "Confirmed" cases, the definitions profess a specific set of lab investigations to be added to the Presumptive diagnosis. However, the present reporting system does not allow the tying up of the presumptively diagnosed cases to their lab reports. Thus, routine weekly surveillance reports provide numbers of syndromic, presumptive and lab-confirmed cases each week, separately.

In 2014, we had conducted a secondary analysis of lab-confirmed infectious

diseases cases reported by IDSP Gujarat. We had found that Enteric Fever, Infectious Hepatitis (A and E) and Dengue cause a high burden of cases in the state (2 to 26 cases per 100,000 population per year), more than that caused by cholera, measles, diphtheria and chikungunya (less than 2 per 100,000 population per year) [17]. Thus, Enteric Fever, Infectious Hepatitis (A and E), and Dengue are being more commonly lab-diagnosed, and in sufficiently large numbers to provide an indication of the capacity in the state for laboratory surveillance of infectious diseases.

Standardized international recommendations from the WHO exist for laboratory diagnoses of the three selected infections. The Indian IDSP follows these recommendations to the letter for acute Infectious Hepatitis and Dengue, but not for Enteric Fever. The challenge to standardization posed by the absence of sensitive, specific, quick and cheap lab methods for Enteric Fever is well documented [18]. The WHO recommends lab diagnosis of Enteric Fever only by isolation of Salmonella spp. from blood or stools [7]. But India's IDSP recommends that the Widal test may be used for establishing a *probable* case; and a four-fold rise of agglutination titre or isolation of *Salmonella typhi* or *paratyphi* establishes a *confirmed* case [16]. For Acute Viral Hepatitis, the WHO recommends that tests for both anti-HAV IgM and surface and/or core antigen for Hepatitis B must be carried out. Once a Non-A and Non-B lab diagnosis is established, the patient must be tested for Hepatitis C, D and E [7]. WHO's guidelines for lab diagnosis of Dengue, published from 2009 to 2012, advocates detection by Rapid Diagnostic Test Kits (RDTKs) for IgM and confirmation by antigen detection through Enzyme Linked Immunosorbent Assay (ELISA), or viral isolation through (PCR) [19]. The IDSP recommendations for diagnosis of probable and confirmatory cases of Acute Viral Hepatitis and Dengue are a reiteration of the WHO guidelines [16].

In this paper, we detail our findings regarding the numbers and capacity of labs in the state of Gujarat for laboratory diagnosis and subsequently, laboratory surveillance of Enteric Fever, Hepatitis (A and E), and Dengue. We discuss the current surveillance methodology adopted by the IDSP and suggest recommendations for the way forward.

2. Material and Methods

Gujarat, with a population of 60.4 million is the 4th most urbanized state in India with 42% of its population living in urban areas [20]. It is subdivided into 33 districts and 248 sub-districts. Being among the urbanized and more developed states in the country, there are many more private labs, than there are public.

We investigated lab capacity in the state in 2015-16 in two stages:

1) Estimation of the number of Labs: This consisted of a survey to assess the number of labs and their distribution across the state.

2) Estimation of the Lab Diagnostic Effectiveness: This consisted of a survey to identify tests conducted in a representative sample of estimated labs, followed by creation of effective diagnostic scores for prevalent tests, grouping tests into

levels, and finally calculating diagnostic capacity.

The concluding results of the evaluation combine these layers to give an overall picture of lab capacity across the state.

2.1. Estimation of Number of Labs

We used publicly available information on locations and levels of health facilities across the state for public labs. Gujarat had approximately 18 Medical Colleges (MCs) (public and private), 24 District Hospitals (DHs), 30 Sub-District Hospitals (SDHs), 363 Community Health Centres—(CHCs), 241 Urban Health Centres (UHCs) and 1194 Primary Health Centres (PHCs).

Of the 18 MCs in the state, eight are state government colleges functioning since more than 50 years, and the others are aided institutions, functioning since a decade or two [21]. For Private labs (**Figure 1**), we obtained a line list of 1307 private labs with telephone numbers, which had been prepared by the Gujarat Department of Health and Family Welfare in June 2013.

The MD-owned (owned by MD or Diploma qualified Pathologists or Microbiologists) labs were located in big cities and the smaller towns had a profusion of Technician-owned labs (graduates with a three years course in Medical Lab Technology (MLT)). Therefore, we divided the Primary list into eight cohorts, MD-owned or Technician-owned labs in four tiers of towns. We then validated this Primary list through physical and telephonic surveys between September to December 2015. During this survey, we also enquired with some of the more responsive labs regarding the conventional testing protocols and methods followed by them as well as names of companies that manufacture the tests used by each of them for Enteric Fever, Hepatitis A and E and Dengue. We also enquired and confirmed the indications for performing tests for each of the diseases during our interviews. Since lab diagnosis of Hepatitis A and E are based on eliminating the diagnosis of Hepatitis B, we also included lab diagnosis of Hepatitis B

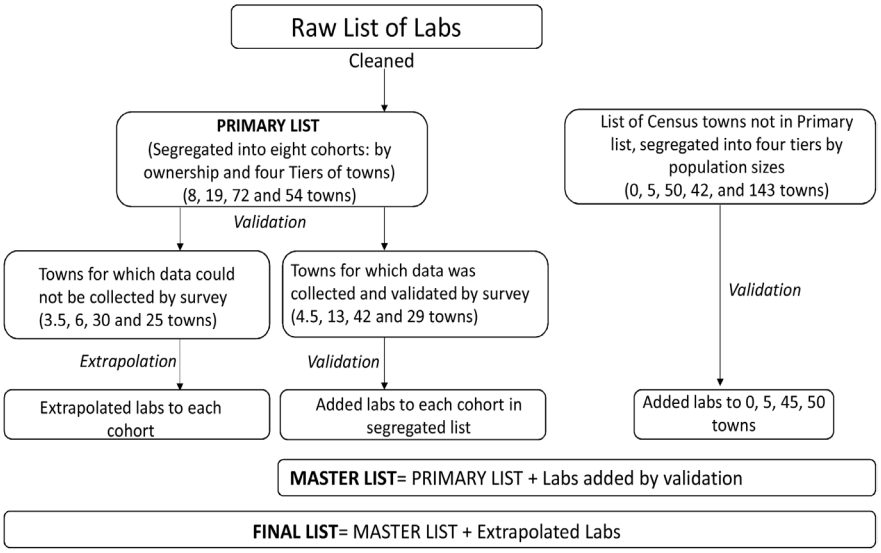

Figure 1. Methodology for estimating numbers of private labs in the state.

in our survey.

We conducted a physical survey of labs in five of the eight Tier 1 cities and added additional labs to the Primary list (**Figure 1**). We paired the remaining three cities with surveyed cities of the same size and extrapolated the numbers in the Primary list for them. For the remaining towns (Tier 2 to 4), we conducted a telephonic survey. Our primary list contained names and telephone numbers of labs in 19 of 24 Tier 2 towns, 72 of 122 tier 3 towns and 54 of 197 Tier 4 towns. We were able to get satisfactory responses from 13, 42 and 29 towns through telephonic survey, thus, we validated (confirmed and added) labs to the primary list. For the remaining 6, 30 and 25 towns where we did not get any telephonic response, we extrapolated the numbers in the primary list by increases we had obtained through the telephonic survey. We did this validation and extrapolation exercise separately for the eight cohorts. For the 5, 50 and 143 towns that were not in the primary list, we conducted telephonic surveys of local governmental health facilities to collect information regarding private labs; 5, 45 and 50 of these towns responded. We built our Master list by adding to the Primary list, all labs that we could confirm for physical existence through phone calls and physical survey. To the Master list, we added the labs we had extrapolated for each tier of towns to create the Final List.

2.2. Estimation of Diagnostic Effectiveness

Our preliminary telephonic survey yielded us the range of lab diagnostic protocols and tests available in all types of labs in the state for Enteric Fever, Hepatitis, and Dengue. Among these, the following constituted the most prevalent algorithms of screening tests being used in the labs to diagnose and treat cases of our selected diseases and were different from internationally recommended guidelines [7] [19] [22] [23].

1) Enteric Fever: Slide and/or Tube Widal tests were universally performed, more often singly than serially. Rarely, newer RDTKs based on market availability such as Typhidot, IgM only, IgG only, IgM/IgG combination kits with the colloidal gold conjugate, etc were done. Culture for *Salmonella* was done, but very infrequently.

2) Hepatitis A and E: Hepatitis B RDTK was first administered to all cases of jaundice of suspected infectious etiology to establish a Non-B diagnosis. Once the case is confirmed to be Non-B, it was then assumed to be Hepatitis A or E by practitioners; further RDTK confirmatory testing for Hepatitis A and E depended on patients' affordability and availability of Hepatitis A/E RDTKs in that lab. ELISA confirmation for Hepatitis A or E was available in major, large government and few private labs.

3) Dengue: NS1 and/or IgM and/or IgG RDTKs were common for suspected Dengue. Some labs did these tests singly and interpreted them as such, while some labs did the tests serially and interpreted them correctly. PCR diagnosis was very rare.

We found that test kits and their principles varied between labs, particularly more for Enteric Fever. We needed a uniform method to assess the effectiveness of these varied tests. Because test accuracies are fundamental to the effectiveness of a lab, we constructed an effective diagnostic score (EDS) for each test using the calculated accuracy of each test obtained from literature. Test accuracy can be calculated from test sensitivity/specificity data and disease prevalence specified in the literature using the formula below [24] [25]. (Table in Appendix)

$$\text{EDS} = \left(\text{Senitivity} - \text{Specificity}\right) * \text{Pretest prob} + \text{Specificity}$$

2.2.1. Mean Lab Diagnostic Effectiveness of Each Cohort

We assessed lab capacity of the eight cohorts of labs through a survey of a sample of labs from each cohort. A detailed survey schedule was administered, based upon the information we had gathered from our preliminary investigations, which asked specifics about the choice of tests, the order of their performance, and manufacturer's name. We visited 50% of all MCs microbiology labs in the state. For private labs, we conducted a telephonic survey of 10% of labs in each of the eight cohorts (by ownership and tier of town) from the Master List. For public labs, we telephonically surveyed 50% of the DH labs, 10% of the CHC labs and all UHC labs in the state.

The score of each lab was the sum of its score for best test for each disease. The overall effectiveness of the cohort therefore was obtained by summing up the effectiveness scores of all effective labs in the cohort.

$$\text{Mean lab diagnostic effectiveness}_{\text{cohort}} = \frac{1}{n}\sum_{i=1}^{n}\text{EDS}_i$$

2.2.2. Levels of Tests

We classified tests into levels based on their accuracies (Table 1). The most accurate, relatively commonly available confirmatory test in the state was considered a Level-3 test/s. Less accurate tests were graded Levels 2 and 1. For Hepatitis A and E, we graded the tests as Level-2 if testing capacity for both Hepatitis A and E was present in the lab and Level-1 if labs tested for either of the diseases,

Table 1. Investigations prevalent in Gujarat for the selected five diseases classified into four levels.

	Level-3 (Gold standard)	Level-2 (EDS ≥ 0.51)	Level-1 (EDS ≤ 0.50)
Enteric Fever	Culture	High accuracy RDTK, Widal Slide + Widal Tube	Widal slide OR tube singly
Hepatitis B	ELISA	Hep B RDTK	Not available
Hepatitis A and E	ELISA	Hep A and E rapid kits or combo kit—both tests available in the lab	Not available
Dengue	PCR, ELISA	Both NS1/IgM RDTK	NS1 alone OR Any non-dual rapid kit

and not for both. Thus, this gave us a normalized cumulative distribution of testing capacity across Levels. Test with EDS > 0.51 were considered level-2 tests and those with EDS < 0.50 were considered level-1 tests. The overall effectiveness of the cohort by levels could also be obtained by calculating the ratio of lab testing at each level to total lab testing for each cohort.

Hepatitis B, A and E did not have any level-1 tests because no other diagnostic tests exist in the field for these viruses. But Dengue and Enteric Fever were clearly being diagnosed by tests with accuracy well below the level-2 tests. Therefore, the inclusion of level-1 tests to the classification was deemed necessary for Enteric Fever and Dengue but not for the Hepatitides.

2.2.3. Ethical Considerations and Conflict of Interest

Ethical approval of this work was obtained from the Institutional Ethics Committee of the Public Health Foundation of India, New Delhi. The authors declare no competing interests, neither in the execution of this research nor in the preparation of this manuscript.

3. Results

3.1. Estimates of Public and Private Labs in the State

We estimated that there are 4293 labs in the state, 1765 public and 2528 private (Table 2). On average, there are 7 labs per 100,000 population in the state. In

Table 2. Summary of public and private labs across tiers of towns.

TIERS: Census classes of cities/towns with population (Number of cities/ towns in this tier)	Proportion of state's population	Private labs			Public labs	Total (% of Total)	Public labs/ Private labs	Labs per 100,000 population
		In Primary list	In Master list	In Final list				
TIER 1: Cities with > 100,000 population and formal Municipal Corporations (8)	26%	515	931	1148	191	1339 (31.2)	0.17	8.53
TIER 2: Towns > 100,000 population but without Municipal Corporation (24)	7%	241	389	417	87	504 (11.7)	0.21	11.92
TIER 3: Towns with 20 - 100,000 population (122)	9%	394	664	731	138	869 (20.3)	0.19	16.0
TIER 4: Towns and villages with <20,000 population (197 towns & 17843 villages)	58%	140	220	232	1349	1581 (36.8)	5.81	4.51
Total population of Gujarat ≈ 60,383,628	100%	1290	2204	2528	1765	4293 (100)	0.70	7.1

Tier 3 towns, this rate more than doubled to 16 labs/100,000 population due to a large number of technician-owned private labs in these towns. However, in Tier 4 towns and villages, in spite of the large number of public labs, it reversed, because of the much larger population in this rural tier.

PUBLIC LABS: Out of 1765 public labs, a large majority, 1150 (67%), were located in Tier 4 towns and villages (in PHCs) (Figure 2). These labs did not conduct tests for our selected diseases and we gave them a dummy score in our analysis to represent redundancy of lab capacity. Of the 24 DHs, 19 had a MD Pathologist and eight had a MD Microbiologist, a few did not have either.

PRIVATE LABS: Nearly half (45%) of the 2528 private labs were located in Tier 1 cities. (Figure 2) MD Pathologists/Microbiologists owned one-third of all private labs, 840; 68% of all MD-owned labs and 34% of all Technician-owned labs were in Tier 1 cities. A significant proportion of Technician-owned labs, 36%, were in Tier 3 towns. These probably also provided diagnostic services to neighboring rural areas. (We mapped 1307 private labs that were provided in the Raw list by the state government as shown in Supplementary Figure 1).

3.2. Pooled Effectiveness of All Labs

The pooled Effective Lab Diagnostic Score (ELDS) of the 4293 labs in the state totaled to 6776. Only 254 (4%) was in the Public lab: MCs, DHs, and CHCs. But it was more evenly distributed across all three levels of testing, compared to the private ELDS. Nearly 80% of the lab effectiveness in the private sector was in level-2 RDTKs (Figure 3).

The contribution to the ELDS by Level-3 confirmatory tests was only 390 out of a total 6776 (6%); the private labs contributed 300 compared to only 90 by the public labs. Since 1415 (265 Private and 1150 Public) labs were not testing for any of our selected diseases, only the remaining 2878 labs in the state contributed

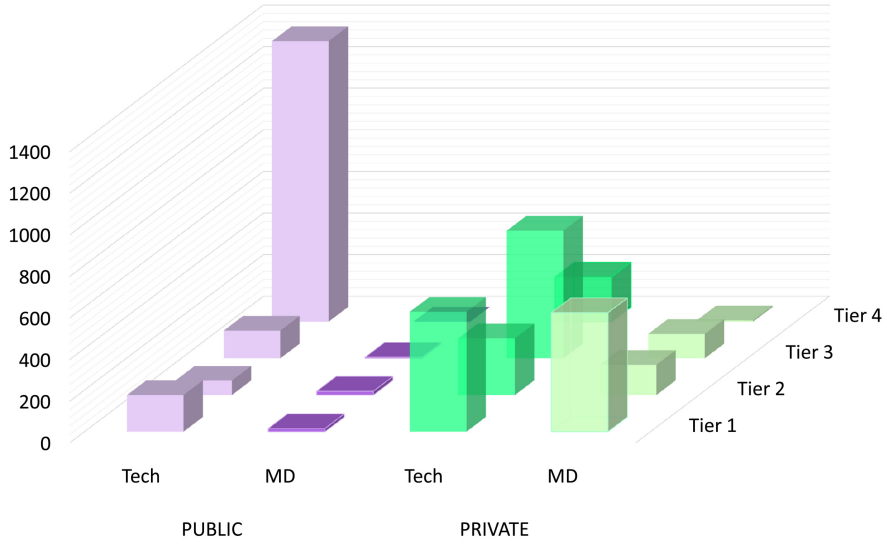

Figure 2. Visual comparative estimate of 4293 MD- and Technician-owned/appointed, private and public labs in four tiers of towns.

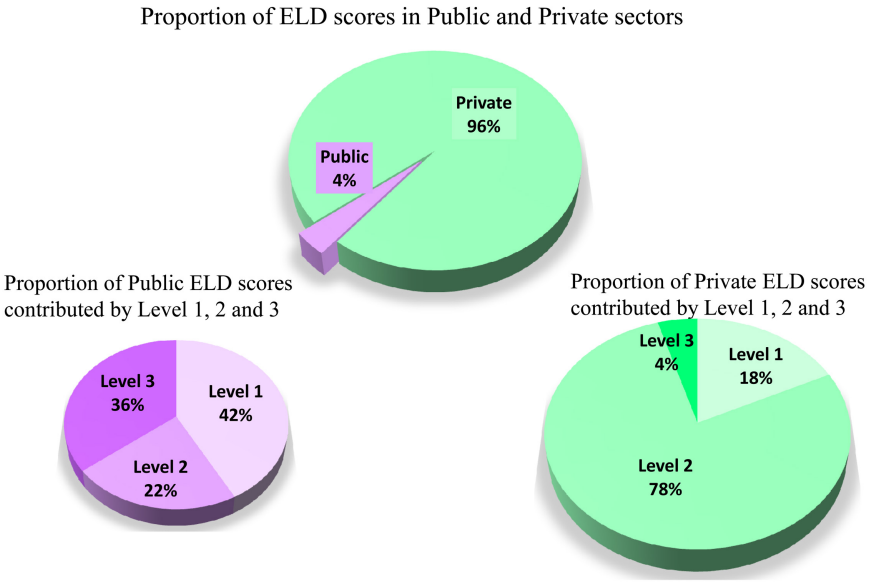

Figure 3. Distribution of pooled ELD scores by Public-Private labs and composition of Public and Private ELDS.

to the pooled score of 6776.

3.3. The Distribution of the Total ELDS in Gujarat by Public and Private Ownership, Location in Tiers of Towns, and Levels of Tests

Laboratory effectiveness was much higher in private than public sector laboratories in all tiers of towns. This over-powering effect was due to two factors, the large numbers of private labs and their predominant usage of level-2 RDTKs.

More than half of the private lab effectiveness was located in Tier 1 cities, and a very small 6% was located in Tier 4 towns. Level-3 confirmatory testing effectiveness was present only in Tier 1 and 2 towns; largely in public sector MCs. Level-0 labs that did not perform one to all of our selected tests were present in both the public and private sector, the largest amount of redundancy being in public sector PHC labs in Tier 4 towns. However, CHCs in these Tier 4 towns performed Level-1 tests.

While 40% of the public lab effectiveness was located in MCs, primarily in Tier 1 and 2 towns, 60% of private lab effectiveness was located in technician-owned labs, primarily in Tier 1 and Tier 3 towns (**Figure 4**).

3.4. Levels of Tests across Diseases

Hepatitis B testing contributed 34% of the total ELDS of 6776, followed by Dengue and Enteric Fever, which contributed 30% and 26% respectively. Hepatitis A and E contributed only 10% of the total ELDS.

Distribution of effectiveness across tiers of towns was most uniform for Enteric Fever and least for Hepatitis A and E. Tier 1 towns possessed nearly 50% of the lab effectiveness for the three larger contributing infections, the remaining

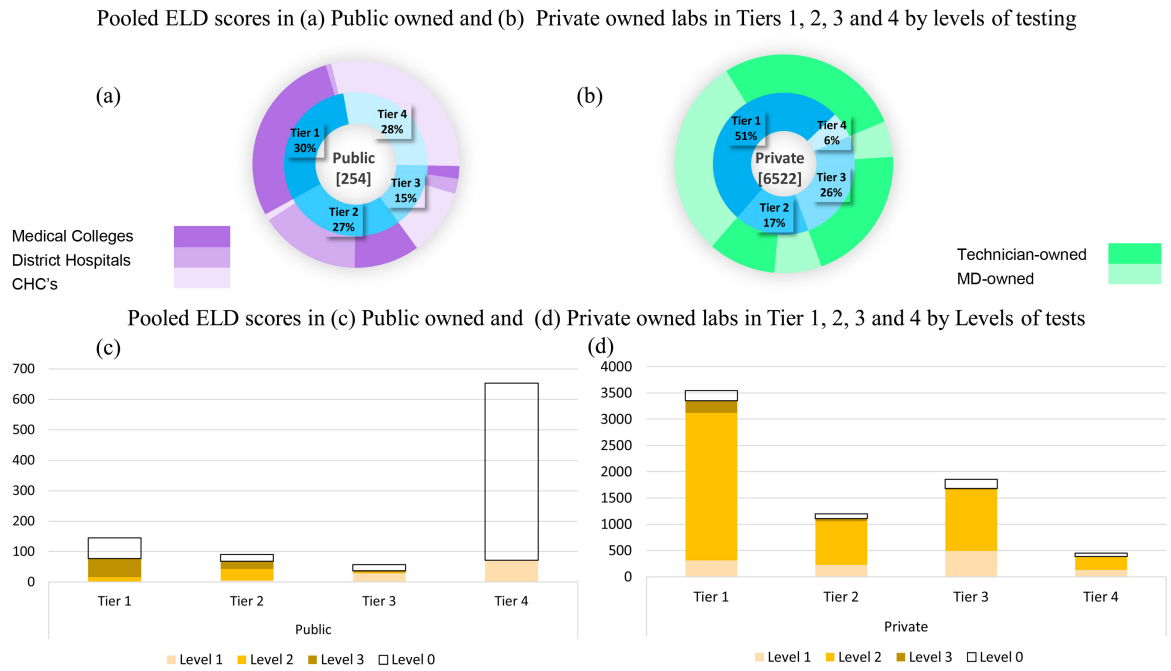

Figure 4. Distribution of ELD scores in public and private labs, and by tiers of towns and levels of testing. [The level-0 bars indicate numbers of labs with no testing for our selected diseases]. Pooled ELD scores in (a) Public owned and (b) Private owned labs in Tiers 1, 2, 3 and 4 by levels of testing. Pooled ELD scores in (c) Public owned and (d) Private owned labs in Tier 1, 2, 3 and 4 by Levels of tests.

effectiveness being distributed in the other tiers. This effectiveness was largely in Level-2 tests and in Technician-owned labs in all four tiers. Level-3 confirmatory testing was present in MD-owned labs for Enteric Fever and Hepatitis B in all three tiers of towns, and for Dengue only in Tier 1 towns. In comparison, level-3 testing by Medical Colleges was negligible. Technician-owned labs mostly performed level-1 testing for Enteric Fever and Dengue in all four tiers of towns (Figure 5).

For Hepatitis A and E, lab effectiveness appeared to be concentrated in Tier 1 towns. Effectiveness score of MD-owned labs in Tier 1 and 2 towns was largely due to RDTKs. Technician-owned labs did not participate in Hepatitis A and E testing as much as they did in the other three diseases. As a result, Level-3 confirmatory testing by MCs and DHs was proportionately more for Hepatitis A and E than for the other infections.

4. Discussion

Our study shows that as much as 94% of lab effectiveness for common and important infectious diseases, in a reasonably well-developed state of India like Gujarat, lies in testing through RDTKs, which are essentially screening tests. Ninety-six percent of the testing capacity is largely located in Tier 1, 2 and 3 cities where 42% of the state's population resides. While there is plentiful availability of RDTKs for Enteric Fever, Hepatitis B and Dengue, it is not so for Hepatitis A and E. Confirmatory testing capacity through cultures for *Salmonella*, and

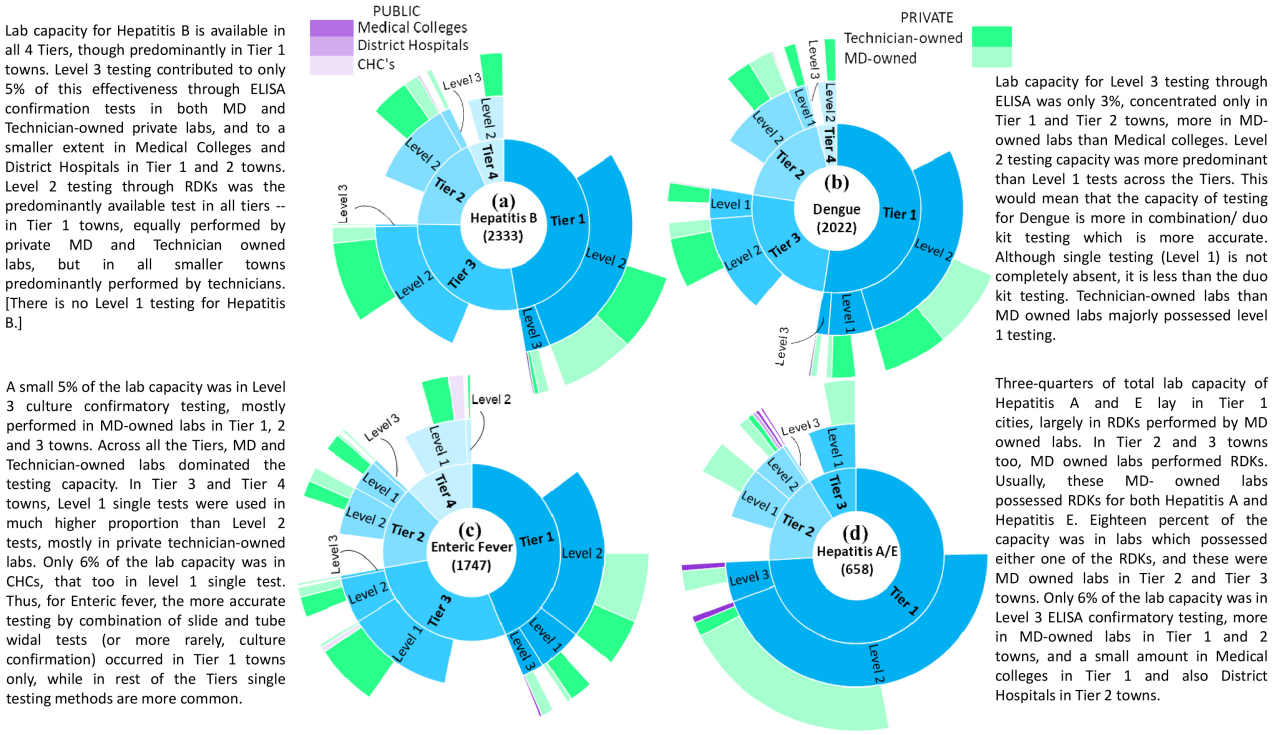

Figure 5. ELDS of each disease contributed by Public-Private laboratories for each Levels and Tiers of towns.

ELISA testing for the viral diseases comprises only 6% of the testing capacity in the state. Although our study was not designed to test the extent of referral linkage practices between screening and confirmatory testing on the ground, it does establish the fact that the capacity in the state is extremely skewed in favor of screening tests.

4.1. Effect of These Large Volumes of Screening Tests Being Used as Confirmatory Diagnostic Tests at the Population Level

4.1.1. A Conservative Estimate of Misdiagnosed Cases

Hepatitis: For the Hepatitides, the very high sensitivity and specificity of Hepatitis B RDTK among symptomatics, means that false positive and negative reports are a very small proportion. Based on the prevalent diagnostic algorithm in the private sector in the state, this would lead to an effective screening-in for the more dangerous Hepatitis B, leaving all the true negatives (Non B cases), to be diagnosed as Hepatitis A or E.

As per the estimations arrived at by Jain et al. in a medical college clinical setting, among 205 consecutive cases presenting with acute viral hepatitis, viral etiology could not be confirmed in 39% of cases. For the 61% cases whose viral etiology could be established, some of them mixed infections, 33% were Hepatitis A and E, 16% Hepatitis B, and 12% were Hepatitis C [26]. Hepatitis A and E are the largest proportion of cases. But since they are self-limiting infections and their treatment is conservative, the absence of confirmatory testing is more a loss for the surveillance system than the individual patient. However, the larger issue

resulting from this local diagnostic protocol is that the lab diagnosis of Hepatitis C and D infections are not being done as recommended by WHO in the private system. This means that the diagnostic protocol currently in use in the private system could be missing as many Hepatitis C cases as it is diagnosing Hepatitis B.

According to the NHP report, around 4000 cases of Viral Hepatitis (all cause) were reported per year in Gujarat in 2016 and 2017, while the Global Burden of Disease project estimates the incidence of all Hepatitis in Gujarat to be 9940 cases per 100,000 population in the same period [27] [28]. This wide difference in estimates of the burden is an obstacle for prioritization of disease burdens and institution of appropriate systemic solutions. It is essential that surveillance systems in the country arrive at more exact estimates of the burden of Hepatitides at state and national levels.

Dengue: The RDTK panel used to diagnose Dengue is reasonably sensitive and specific. Shepard *et al.* estimated that the annual average clinically diagnosed case of Dengue in India was 5.8 million cases, 282 times more than the officially reported annual average for 2006 to 2012. Half of these, 3 million, underwent a panel of NS1, IgG, and IgM. But since our systems are alleged to be reporting a very tiny proportion (200 to 300 times less), of these as confirmed cases, there is presumably a large underestimation of the burden of Dengue in the country. Since the treatment of Dengue depends more on clinical presentation and platelet counts, and not much on the RDTK reports, the moderate numbers of false positives and negatives would not change clinical course of individual cases. However, if this testing could be captured authentically by the IDSP from a representative group of urban, semi-urban and rural labs, and could be linked to PCR confirmation in a pre-decided proportion of cases, the true burden of Dengue and its transmission trends over the years would be available to planners.

Enteric Fever: The effects of false positives due to Slide Widals for Enteric Fever are much more alarming. A study on 100 pediatric subjects, clinically suspected to be suffering from Typhoid in a tertiary hospital in Delhi found that only seven of them were truly positive for typhoid with both Widal and blood culture positive. Forty-two were clinically consistent with typhoid symptoms and Widal positive. However, all the 100 were administered ceftriaxone before receipt of Widal test results [29].

With a specificity of 47.31% and sensitivity of 71.43% [29], for every 100,000 true positive tests reported by Slide Widal, 49,000 false positive patients (nearly half as many), could potentially be treated with a course of higher antibiotics. Misuse and overuse of antibiotics pose an ever increasing threat of severe infections due to antibiotic resistant organisms, increased complications, longer hospital stays and increased mortality [30]. Treating false positives with antibiotics adds antibiotic resistant typhoidalsal monella strains to the environment [31].

4.1.2. A Conservative Estimate of Cost of Misdiagnosis

Our earlier calculation of burden of laboratory confirmed cases of these infec-

tions reported in Gujarat and India, had shown that Enteric Fever and Hepatitis A&E are diagnosed at least 10 times more than cases of Dengue. (Table 3) Based on this, we made a conservative estimate of the annual expenditure on only the tests reported positive into the government system from 2006 to 2011. Gujarat spent INR 10million and India spent INR 295 million on *only* positive tests.

Shepard *et al.* estimated that Dengue testing using a panel of NS1, IgG, and IgM was done in 3 million cases in India every year from 2006 to 2012 [33]. We can assume that use of RDTKs for Enteric Fever and Viral Hepatitis must be more voluminous than this because ten times more lab-confirmed cases of these infections are being reported [27]. For 3 million to 30 million RDKTs for these three infections, India would have spent $108.7 million to $1087 million (2012 value of USD). India's central expenditure allocation on all vector-borne diseases in the 12th Five Year Plan, at $526 million, was within this range [34]. Therefore, the Indian public probably spends many more times on just RDTKs for common infectious diseases, than the state is able to muster for control of these very diseases.

4.2. Way Forward

The Integrated Diseases Surveillance Project (IDSP) in Gujarat collects lab reports from 2563 lab reporting units at present, only 720 of them being from the private sector *i.e.* 28% of the private labs estimated by us [35]. As a result, although a large number of RDTKs are conducted in the state, these are not reflected in the data captured by the surveillance system. The IDSP must recruit representative private labs carefully into their reporting system. There is also a need to widely publicize the WHO recommended laboratory diagnostic protocol for these three common infections among the public and private lab owners, particularly technician-owned labs in Tier 3 towns in the state.

Inspite of the very large volumes of trade in RDTKs in endemic countries, [36] there have not been any concerted efforts to extract epidemiologically useful data from them. A planned sampling of RDTK positive and negative cases for PCR or ELISA confirmation could be used to create a map of regional burdens of these infections. Particularly in case of dengue, the ongoing transmission of

Table 3. Summary of case rates and expenditure per year over RDTK testing in India.

Diseases	Case Rates (per 100,000/year)			Cost of RDTK (INR) [32]	Expenditure per year (in million INR)		
	Gujarat IDSP [17]	India CBHI [17]	Shepard *et al.* estimates [33]		Gujarat IDSP	India CBHI	Shepard *et al.* estimates
Dengue	2.46	1.27	3 mill	1800	2.67	28.35	5400
Hepatitis A & E	24.76	10.85	3 mill	500	7.48	67.27	1500
Enteric Fever	26.71	80.57	3 mill	200	3.22	199.81	600
Total (million INR)					10.15	295.43	75
Total (million $)					0.15	4.24	108.70

the virus could be mapped. This could be used strategically to prevent disease transmission into semi-urban and rural India. The large logistics network of RDTK manufacturers and retailers could be definitely encouraged to produce such data, which could inform state and national governments of regional burdens of these diseases, and even transmission pathways of diseases like Dengue.

For the clinical and lab units that do report into the IDSP, the design of the system does not allow linkage of clinical (Probable) and lab diagnoses of these infections. The present design of IDSP's data collection forms does not allow estimation of the true burden of these diseases. The "P" (Probable diagnosis) and "L" (Lab diagnosis) forms are not coordinated with each other according to the definitions. More importantly, the "L" form is not designed to capture the most prevalent lab diagnostic algorithms through screening tests being used in the community to diagnose and treat cases. The probable and lab diagnosed cases are collected independently. It is time that the IDSP designed the forms in a strategically *nested* manner to collect RDTK report and confirmatory (ELISA and/or Culture) report in the "L" forms of cases with a probable diagnosis reported in "P" forms.

With a representative sample of private lab reporting units, "L" form nested in the "P" form, and strategically planned confirmatory testing, it would be possible for the IDSP to begin mapping the true prevalence of these infections across various districts and sub-districts in the state. Although understaffed and under-funded, the IDSP is presently the best placed and equipped organization to carry out this exercise [37]. Over time, as the IDSP becomes robust and reliable, it may be in a position to improve the level of confirmatory testing in private labs too.

4.3. Strengths and Limitations of This Study

Our estimations of numbers of labs and their effectiveness are conservative. The time frame and finances of our study did not allow for a physical survey of the entire state for labs. The number of labs in the state may be more than our estimate, not less. The larger proportion of technician owned labs in the state, mostly located in smaller towns might be more than our estimate, not less. We used a "head-count" of labs. This treats both large medical college labs, handling hundreds of samples in a day, as well as single room clinics in remote areas, handling a few samples a month, as one lab. We overcame this through the construction of ELDS. Since our objective was to understand the effectiveness of labs in the diagnosis of endemic infections and their distribution across the state, the numbers of samples each processed was not as important as the extent to which each lab specialized in higher level testing for each of the diseases. The assignment of effectiveness scores to each lab for diagnosis of each disease gave us an indication of the level of testing for each disease in each of the labs, and collectively when grouped by Tiers, ownership or disease. These effectiveness scores are also conservative as it assumes that a lab would use the most accurate test it

has for a given disease. However, this is not always the case especially in the private sector where patients' affordability is an important factor. Therefore, it is important to recognize that our findings do not account for volumes of samples tested in each lab or extent of confirmatory testing actually done on the ground. It is only an estimate of existing lab capacity for testing of endemic infectious diseases. However, to our knowledge, this is the first attempt at documenting and quantifying the landscape of surveillance for a handful of Neglected Tropical Diseases in a moderately large region in the world.

The sensitivity, specificity, and prevalences used to determine the Effective Lab Diagnostic Scores in this study are based on broad prevalence of the diseases in Kolkata in West Bengal (Enteric Fever) [38], Lucknow in Uttar Pradesh (Hepatitides) [26] and city of Ahmedabad (Dengue), as gathered from literature. There is little evidence of any such testing studies being conducted in India, and none in Gujarat. Therefore, the accuracy of the scores may be subject to change once more accurate information is available. Since the score is constructed to be prevalence dependent, we believe it may represent a more accurate factor to use as compared to F1 score and G scores that do not account for prevalence when being calculated. The estimation of ELDS also assumes that the population of Gujarat is homogenous with regard to its response to each of the tests.

5. Conclusions

Our study has established the size and capacity of the private and public lab network in the state of Gujarat. Given the fact that we depend heavily on Rapid Kits to diagnose important endemic infections as well as many other infectious and non-infectious conditions, and that this diagnostic method is growing very fast, we need to establish the true accuracies of these tests across regions. The disquieting issue uncovered by this study is the very dismal availability of confirmatory testing in the large and dominant private sector. The large-scale acceptance of screening tests as diagnostic tests also indicates a lack of professionalism among practitioners in the state.

We need to design data collection, collation and confirmatory testing strategies that can be implemented by the IDSP program through the public and private lab networks to calculate the effectiveness of the diagnostic tests at varying prevalences in the country. This would be the quickest method to estimate the burden of, and accordingly, prioritize responses to endemic infections in the country.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Table A1. List of pre-test probabilities for selected diseases among populations with symptom complexes that qualify the use of their respective diagnostic tests.

Disease	Indications	Pretest prob	Test name	Sensitivity	Specificity	EDS
Typhoid	Fever cases that persist for 2 days	0.0280 [38]	Slide Widal [O] [39]	0.952	0.036	0.061648
			Slide Widal [H] [39]	0.803	0.5	0.508484
			Single tube Widal [O] [39]	0.873	0.069	0.091512
			Single tube Widal [H] [39]	0.952	0.138	0.160792
			Magnetic Semi-quantitative immunoassay IgM [39]	0.73	0.69	0.69112
			IgM only [39]	0.75	0.607	0.611004
			IgG only [39]	0.692	0.704	0.703664
			IgM/IgG combo	0.75	0.704	0.705288
			IgM/IgG combo with colloidal gold conjugate	0.8545	0.8861	0.885215
			Serial (Slide + Tube) Widal	0.764456	0.569	0.574473
Dengue*	High fever, myalgia and headaches presenting after rain	0.3449 [40]	Dengue IgM	0.8	0.970811	0.911898
			**Dengue NS1 RDTK [41]	0.7676	0.9831	0.908774
			Dual NS1/IgM [37]	0.8865	0.9875	0.952665
Hepatitis B	Acute illness of <15 days with symptoms of Jaundice	0.1610 [26]	Hepa card [42]	1	0.9874	0.989429
Hepatitis A		0.2696 [26]	CTK HAV IgM	0.995	1	0.998652
Hepatitis E		0.1797 [26]	CTK HEV IgM	1	0.993	0.994258

**NS1 RDTK test for Dengue is effective if tested within 2 days of onset of symptoms. *PCR is available for Dengue, but is expensive and very, very rarely used. Therefore not considered in our study. EDS.

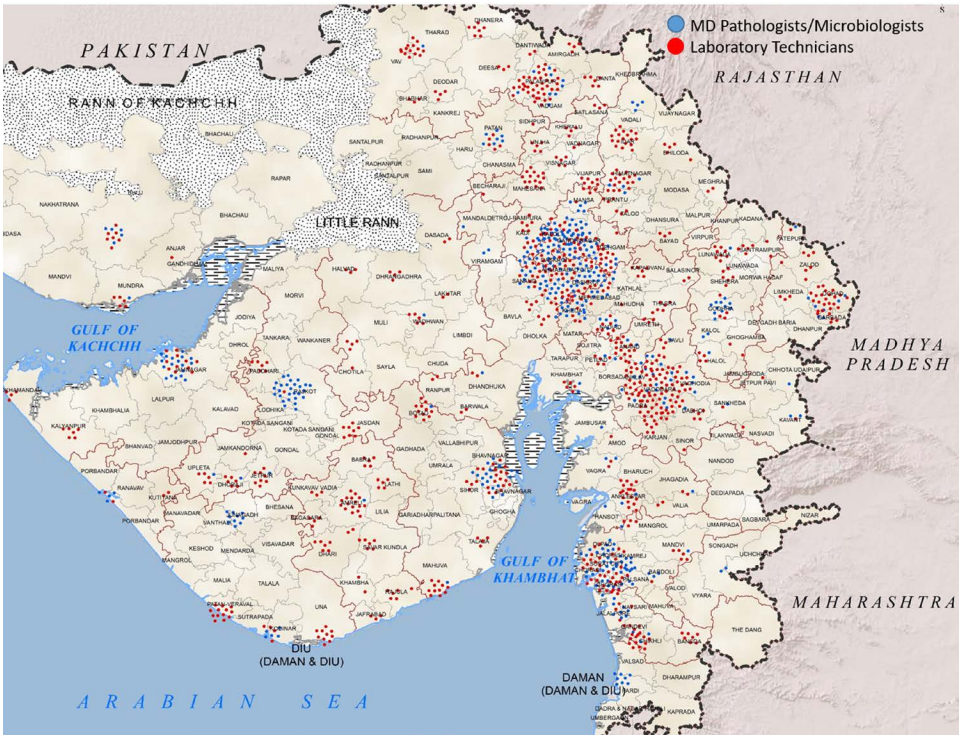

Figure A1. Distribution of private labs in Gujarat based on raw list of 1307 private labs.