TITLE: Health Technology Assessment of various RT-PCR kits/methods for the diagnosis of Influenza A/H1N1pdm09 virus in all age group patients in India.

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List of Abbreviations

AIIMS	All India Institute of Medical Sciences
CDC	Centers for Disease Control and Prevention
DHR	Department of Health Research
FN	False Negative
FP	False Positive
GOI	Government of India
HA	Haemagglutinin
HTA	Health Technology Assessment
ICMR	Indian council of Medical Research
ILI	Influenza-like illness
INR	Indian Rupee
IPD	In Patient Department
NA	Neuraminidase
NCDC	National Centre for Disease Control
NIV	National Institute of Virology
NPV	negative predictive values
OPD	Out Patient Department
PICOS	Population - intervention - comparator - outcomes - study design
PPV	Positive predictive values
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-analyses
RIDT	Rapid Influenza Diagnostic tests
rtRT-PCR	Real-time reverse transcriptase polymerase chain reaction
TN	True Negative
TP	True Positive
VRDL	Viral Research & Diagnostic Laboratories
WHO	World Health Organization

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Executive Summary

Lower respiratory tract viral infections, including those due to influenza, are among the most common infectious diseases in humans and they are associated with significant morbidity and mortality. Seasonal influenza viruses infect 5–15% of the human population each year, resulting in ~500,000 deaths worldwide. Influenza is vaccine-preventable and antiviral treatment is available. A significant number of severely ill patients infected with H1N1pdm09 requiring intensive care and mechanical ventilation for severe viral pneumonia.

Nucleic acid tests are sensitive and specific and provide a rapid diagnosis, making them invaluable for patient and outbreak management. Real-time polymerase chain reaction (PCR) can be considered as gold standard for detection of influenza viruses due to its high sensitivity and specificity. Real time RT-PCR developed by WHO is considered as gold standard method for influenza A H1N1pdm09 diagnosis from Nasal/throat swabs.

Currently in India, suspected patients are screened by clinician and prescribing Oseltamavir drug without waiting for test report. Government of India recommended testing of samples from suspected patients of category C only. Indiscriminate use of anti influenza drug may develop resistance in virus. Limited labs are testing H1N1pdm09 virus in country and have not been able to effectively implement at large programs due to lack of adequate infrastructure, trained manpower and limited resources and high costing of kits. Sensitivity, specificity and cost of different tests have huge variation. The purpose of this assessment was to appraise the current evidence for the clinical effectiveness (in terms of sensitivity and specificity) and costing of kits against CDC/WHO real time RT-PCR for diagnosis of influenza A/H1N1pdm09 in India.

Sensitivity and specificity was obtained from kits (n=4) evaluated at NIV. Thermo Fisher Scientific (Invitrogen) Pandemic H1N1/09 Assay kit sensitivity and specificity for Influenza A target of A(H1N1)pdm09 virus was 100% (95% CI: 91.8, 100) and 100% (95% CI: 96.53, 100) respectively. Sensitivity and specificity for Influenza Hemagglutinin H1 target of A/H1N1pdm09 virus was 100% (95% CI: 91.8, 100) and 100% (95% CI: 96.53, 100) respectively. Sensitivity and specificity for Influenza A target of A (H3N2) virus was 100% (95% CI: 85.69, 100) and 100% (95% CI: 97.06, 100) respectively.

TRUPCR H1N1 kit (3BBlackBio, Bhopal) sensitivity and specificity for Influenza A target was 100% (95% CI: 95.06, 100) and 94.87% (95% CI: 87.54, 97.99) respectively. Sensitivity and specificity for Influenza H1 target of A(H1N1)pdm09 virus was 94.44% (95% CI: 84.89, 98.09) and 100% (95% CI: 96.23, 100) respectively.

Qiagen artus Infl./H1 LC/RG RT-PCR Kit sensitivity and specificity for Influenza target was 84.78% (95% CI: 76.06, 90.71) and 96.61% (95% CI: 88.46, 99.07) respectively. Sensitivity and specificity for Influenza H1 target of A(H1N1)pdm09 virus was 94.55% (95% CI: 85.15, 98.13) and 98.96% (95% CI: 94.33, 99.82) respectively.

Cepheid Xpert® Flu kit **s**ensitivity and specificity for FluA target of A(H1N1)09pdm virus tested was 100% (95% CIs: 88.65, 100) and 99% (95% CIs: 95.39, 99.85) respectively. Sensitivity and specificity for influenza hemagglutinin H1 target of A/H1N1pdm09 virus tested was 93.33% (95% CIs: 78.68, 98.15) and 100% (95% CIs: 96.87, 100) respectively. Sensitivity and specificity for Influenza B virus was 96.67% (95% CIs: 83.33, 99.41) and 100% (95% CIs: 96.87, 100) respectively. Sensitivity and specificity for Flu A target of A (H3N2) virus tested was 63% (95% CIs: 38.64, 81.52) and 100% (95% CIs: 97.19, 100) respectively.

Influenza A/H1N1pdm09 testing cost (Rs) per sample for invitrogen kit was 2015, Qiagen kit 1902, TruPCR kit 1660, and Cepheid kit 4342. In view of highest sensitivity and specificity among all the kits evaluated in this study, Invitrogen kit is recommended for diagnosis of influenza A/H1N1pdm09 virus from clinical samples.

1. BACKGROUND:

Lower respiratory tract viral infections, including those due to influenza, are among the most common infectious diseases in humans and they are associated with significant morbidity and mortality [Lim et al 2016]. Despite decades of surveillance and pharmaceutical and non-pharmaceutical interventions, seasonal influenza viruses continue to cause epidemics around the world each year. Seasonal influenza viruses infect 5–15% of the human population each year, resulting in ~500,000 deaths worldwide [Stohr K 2002]. The annual recurrence of seasonal epidemics is attributed to the continued evolution of seasonal influenza viruses, which enables them to escape the immunity that is induced by prior infections or vaccination, and to the ability of those viruses to be transmitted efficiently from human-to-human via respiratory droplets, direct contact and fomites. Influenza is vaccine-preventable and effective in preventing the spread of seasonal influenza virus epidemics, but they must be updated regularly to keep pace with the evolution of the circulating viruses.

Influenza viruses belong to the *Orthomyxoviridae* family and are divided into types A, B, C and D. Influenza types A and B are responsible for epidemics of respiratory illness that are often associated with increased rates of hospitalization and death. Influenza type A and B viruses have 8 genes that code for 11 proteins, including the surface proteins haemagglutinin (HA) and neuraminidase (NA). Influenza A viruses are further classified into subtypes based on the combination of haemagglutinin (HA) and neuraminidase (NA) glycoproteins on their surfaces. To date, 18 HA subtypes and 11 NA subtypes have been identified.

The hallmark of human influenza viruses is their ability to undergo antigenic change, which occurs by two ways: **Antigenic drift** and **Antigenic shift.**

Antigenic drift– is a process of gradual and relatively continuous change in the viral HA and NA proteins. It results from the accumulation of point mutations in the HA and NA genes during viral replication. Both influenza type A and B viruses undergo antigenic drift, leading to new virus strains. The emergence of these new strains necessitates the frequent updating of influenza vaccine virus strains. Because antibodies to previous influenza infections may not provide full protection against the new strains resulting from antigenic drift, individuals can have many influenza infections over a lifetime.

Antigenic shift– in addition to antigenic drift, influenza type A viruses can also undergo a infrequently and unpredictably type of change called antigenic shift. A shift has occurred

when an influenza type A virus emerges among humans bearing either a HA protein or a combination of HA and NA proteins that have not been circulating among humans in recent years.

In India, peaks of influenza were observed during July-September coinciding with monsoon in cities Delhi and Lucknow (north), Pune (west), Allaphuza (southwest), Nagpur (central), Kolkata (east) and Dibrugarh (northeast), whereas Chennai and Vellore (southeast) revealed peaks in October-November, coinciding with the return monsoon months in these cities. In Srinagar (Northern most city at 34°N latitude) influenza circulation peaked in January-March in winter months (Kaul et al 2014, Chadha et al 2015).

Antiviral treatment is available and effective for influenza if the disease is identified early in the course of illness, so rapid and accurate laboratory diagnosis is particularly important in both the inpatient and outpatient settings. The rapid and accurate diagnosis of the underlying pathogen is crucial for establishing good clinical practices aimed at reducing morbidity and mortality. In addition, knowing the etiologic agent of these infections can result in significant improvement in patient management by permitting the judicious use of antiviral agents in an era where antiviral resistance is continuing to increase. Epidemics of influenza occur almost every year in temperate climates, the rates and severity of illness caused can vary substantially from year to year. The severity of annual epidemics is affected by several factors including the types, subtypes and strains of circulating viruses, and the level of protective antibodies in the general population.

Nair et al (2013) estimated that in 2010, 11·9 million (95% CI 10·3-13·9 million) episodes of severe and 3·0 million (2·1-4·2 million) episodes of very severe ALRI resulted in hospital admissions in young children worldwide. They estimated that roughly 265,000 (95% CI 160,000-450,000) in-hospital deaths took place in young children, with 99% of these deaths in developing countries.

Influenza was associated with 10% (95% CI 8%-11%) of respiratory hospitalizations in children <18 y worldwide, ranging from 5% (95% CI 3%-7%) among children <6 mo to 16% (95% CI 14%-20%) among children 5-17 y (Lafond et al 2016). On average, estimated influenza results in approximately 374,000 (95% CI 264,000 to 539,000) hospitalizations in children <1 y-of which 228,000 (95% CI 150,000 to 344,000) occur in children <6 mo-and

870,000 (95% CI 610,000 to 1,237,000) hospitalizations in children <5 y annually. Influenza-associated hospitalization rates were more than three times higher in developing countries than in industrialized countries (150/100,000 children/year versus 48/100,000) (Lafond et al 2016).

1.2 Epidemiology: Indian Scenario

A significant number of severely ill patients infected with H1N1pdm09 requiring intensive care and mechanical ventilation for severe viral pneumonia. Pandemic H1N1 virus had significantly higher risk of hospitalization than those positive for seasonal influenza-A viruses (Mishra et al 2010). The rapidity with which the pandemic (H1N1) 2009 virus spread highlighted the need for timely and effective surveillance systems to detect emerging viruses with pandemic potential, and the need for standard platforms for data sharing and dissemination.

Influenza surveillance was carried out in patients with influenza-like illness (ILI) presenting at All India Institute of Medical Sciences (AIIMS), New Delhi. Of the 3264 samples tested, 541 (17%) were positive for influenza viruses, of which 221 (41%) were pandemic Influenza A(H1N1)pdm09, 168 (31%) were seasonal influenza A, and 152 (28%) were influenza B (Broor et al 2012). Influenza A/H1N1pdm09 and influenza B were found in 58% and 42% samples respectively from November 2012 to Feb 2013 in Kashmir, India (Koul et al., 2013)

Population-based active surveillance in India showed higher incidence rates for influenza A(H1N1)pdm09 among children during pandemic versus postpandemic periods (345 vs. 199/1,000 person-years), whereas adults had higher rates during postpandemic versus pandemic periods (131 vs. 69/1,000 person-years) (Broor et al 2012).

Chadha et al (2013) conducted a population-based study to estimate the incidence of laboratory confirmed influenza-associated hospitalizations in a rural community in Pune, western India during pandemic and post pandemic periods (May 2009-April 2011). Among 9,426 hospitalizations, 3,391 (36%) patients were enrolled; 665 of 3,179 (20.9%) tested positive for influenza. Of 665 influenza positives, 340 (51%) were pandemic A(H1N1)pdm09 and 327 (49%) were seasonal, including A/H3 (16%), A/H1 (3%) and influenza B (30%).The proportion of patients with influenza peaked during August 2009 (39%) and 2010 (42%). The adjusted annual incidence of influenza hospitalizations was 46.8/10,000 during pandemic and

40.5/10,000 during post-pandemic period with comparable incidence of A(H1N1)pdm09 during both periods (18.8 and 20.3, respectively). The incidence of both H1N1pdm09 virus and seasonal hospitalized influenza disease was highest in the 5-29 year olds.

Hirve et al (2015) conducted a multi-site population-based surveillance study to estimate and compare rates of influenza-associated hospitalization at Ballabgarh and Vadu during 2010-2012. Healthcare utilization surveys (HUS) showed that 69% and 67% of hospitalizations occurred at study facilities at Ballabgarh and Vadu, respectively. The proportion of patients with influenza was higher at Vadu than Ballabgarh annually (2010: 21% vs. 5%, $p < 0.05$; 2011: 18% vs. 5%, $p < 0.05$; 2012: 23% vs. 5%, $p < 0.05$). Annual adjusted influenza-associated hospitalization rates were 5-11 fold higher in Vadu (20.3-51.6 per 10,000) vs Ballabgarh (4.4-6.3 per 10,000). At both sites, influenza A/H1N1pdm09 and B predominated during 2010, A/H3N2 and B during 2011, and A/H1N1pdm09 and B during 2012.

1.3 Diagnosis and Treatment methods available in India

Nucleic acid tests are sensitive and specific and provide a rapid diagnosis, making them invaluable for patient and outbreak management. Real-time polymerase chain reaction (PCR) can be considered the gold standard for detection of influenza viruses due to its high sensitivity and specificity. Real time RT-PCR developed by WHO is considered as gold standard method for influenza A H1N1pdm09 diagnosis from Nasal/throat swabs.

In India, all the central and state govt labs are testing suspected H1N1pdm09 cases using real time RT-PCR test using H1N1pdm09 assay kit developed by WHO collaborating centre CDC Atlanta USA and licensed to Invitrogen BioServices for manufacturing and marketing. Newer version of CDC kit developed in 2015 and licensed to Integrated DNA Technologies for manufacturing. Whenever required, National Institute of Virology, Pune or NCDC, Delhi centrally procure the required reagents (H1N1pdm09 assay kit, RT-PCR kit, Nucleic acid extraction kit and viral transport media) and distributing to the NIV, VRDL and NCDC network central govt labs. State govt labs and private labs are procuring reagents at their own. In India, all the states have Influenza testing facility by real time RT-PCR.

All individuals seeking consultations for flu-like symptoms screened at healthcare facilities, both Government and private or examined by a doctor and categorized into A, B and C. In order to prevent and contain outbreaks of Influenza, the following guidelines for screening, testing and isolation are to be followed (https://ncdc.gov.in/showfile.php?lid=361):

Category- A (uncomplicated/mild):

- Symptomatology: Patients with mild fever and cough/ sore throat with or without body aches, headache, diarrhea and vomiting will be categorized as Category-A.
- Diagnostic test: Testing of such patients (Category-A) for Influenza is not required
- Treatment: They do not require Oseltamivir and should be treated for the symptoms mentioned above. The patients should be monitored for their progress and reassessed at 24 to 48 hours by the doctor.
- Isolation: Patients should confine themselves at home and avoid mixing up with public and high-risk members in the family

Category-B (uncomplicated but severe symptoms / high risk groups):

- In addition to all the signs and symptoms mentioned under Category-A, if the patient has high grade fever (≥102 F) and severe sore throat
- In addition to all the signs and symptoms mentioned under Category-A, individuals having one or more of the following high-risk conditions
    1. Age ≥ 65 years
    2. Pregnancy (including up to two weeks post-partum)
    3. Infants and Children aged ≤ 5 years (especially <2 years of age)
    4. Chronic respiratory disease
    5. Chronic heart, kidney, liver or neurological disease
    6. Diabetes mellitus
    7. Blood disorders (including haemoglobinopathies)
    8. Persons with immunosuppression (including HIV/ AIDS & use of long term (≥2 weeks) corticosteroids, Post-transplant patients)
    9. Extreme obesity (BMI ≥ 40 kg/m^2)
    10. Malignancy

- Diagnostic test: Testing of the Category-B patient for Influenza is not required.
- Treatment: They should receive Oseltamivir along with symptomatic treatment.
- Isolation: All patients of Category-B should confine themselves at home and avoid mixing with public and high-risk members in the family.

Category-C (Complicated):

- Symptomatology: In addition to the above signs and symptoms of Category-A and Category-B, if the patient has one or more of the following: **Symptoms and signs of complicated influenza Symptoms**

Symptoms	Signs
Breathlessness Hemoptysis Altered mental status Somnolence and Poor feeding (in children) Seizures Decreased urine output Persistence or worsening of initial symptoms beyond 72 hours Worsening of underlying chronic conditions like Diabetes Mellitus, Chronic Kidney Disease etc.	Tachypnoea SpO2<90% Hypotension Reduced urine output Cyanosis

- **Diagnostic test:** These patients should be tested for influenza; start empirical antiviral therapy (oseltamivir) while results are pending
- **Treatment:** immediate hospitalization and treatment.

2. Rational of the study:

Indiscrimine use of anti influenza drug may develop resistance in virus. Limited labs are testing H1N1pdm09 virus in country and have not been able to effectively implement at large programs due to lack of adequate infrastructure, trained manpower and limited resources and high costing of kits. Sensitivity, specificity and cost of different molecular tests exhibits huge variation. The purpose of this assessment was to appraise the current evidence for the clinical effectiveness (in terms of sensitivity and specificity) and cost-effectiveness of different RT-PCR kits against CDC/WHO real time RT-PCR for diagnosis of influenza A/H1N1pdm09 in India.

2.1 POLICY QUESTION:

Which RT-PCR based test should be used as diagnostic test for H1N1pdm09 virus in India?

2.2 RESEARCH QUESTION:

1. What is the diagnostic effectiveness and which is cost effective testing strategy/protocol for diagnosis of Influenza A/H1N1pdm09 virus in India?
2. What will be the benefit of introduction of Influenza A/H1N1pdm09 virus diagnosis in India medical management or/and in public health decision-making**?**
3. How feasible is introduction of large scale Influenza A/H1N1pdm09 virus diagnostic program in India?

2.3 AIM & OBJECTIVES:

1. To assess sensitivity and specificity of various real time RT-PCR assays using as a diagnostic test for H1N1pdm09 virus in India over CDC real time RT-PCR.
2. To assess the cost-effectiveness of the various real time RT-PCR assays using as a diagnostic test for H1N1pdm09 virus in India over CDC real time RT-PCR.
3. To study the turnaround time (speed of diagnosis) of various diagnostic tests.
4. To assess the equity aspects of introducing RT-PCR method for H1N1 diagnosis as standard practice.

3. METHODOLOGY:

PICO criteria used for study were as given below:

3.1 PICO

- Population: All age group patients with influenza like illness.
- Intervention: Real time RT-PCR based methods/commercial kits
- Comparator: Centre for Disease Control and Prevention (CDC) protocol as a gold standard.
- Outcomes: Clinical effectiveness (sensitivity & specificity), diagnostic accuracy and cost effectiveness of real time RT-PCR based methods/commercial kits.

3.2 Clinical Effectiveness (Sensitivity & Specificity) Literature Review

The research methodology was designed using PRISMA-P (Preferred Reporting Items for Systematic reviews and Meta-analyses Protocols) statement guidelines including the preparation of a pre-specified protocol and analysis plan.

1. **Databases & Sources:**

   Comprehensive electronic searches were undertaken to identify relevant reports of published studies from April 2009 to assess the diagnostic performance (in terms of sensitivity and specificity) and cost-effectiveness of the test. Major electronic databases searched were Pubmed, EMBASE, Wiley Online Library, Pubmed Central (PMC), U.S. National Library of Medicine (NLM), Cochrane Database of Systematic Reviews (CDSR), Centre for Reviews & Dissemination (CRD-NIHR) including Database of Abstracts of Reviews of Effects (DARE) Health Technology Assessment Database (HTA) and NHS Economic Evaluation database (NHS EED). In addition to literature review from electronic databases, efforts have also been made to collect relevant information from manufacturer's website including their reports and supplementary data related to their products.

2. **Electronic Search Strategy:**

   Keeping in view the research question, specific keywords were selected and strategies were made using conjunctions and linking words like 'AND', 'OR', 'NOT'. Various combinations of keywords and conjunctions were attempted applying a range of search filters like article type, date range searched; availability of text and species etc. and the strategy giving maximum relevant output was finally selected. An individual search strategy was made for each electronic database searched. The electronic

databases were last searched on 13 August 2019. The final search strategy selected to perform Pubmed database search is given in the **Table 1**.

3. **<u>Study Selection</u>:** The results/outcomes of the search conducted using different databases were further selected on the basis of Inclusion and Exclusion criteria designed at the time of protocol preparation. Studies were selected for inclusion/exclusion through a two-stage process as illustrated in PRISMA flowchart **(Fig 1)**. The inclusion and exclusion criteria opted for the study selection was as follows:

    - **Inclusion criteria:** The studies were included on the basis of PICOS design which includes the following criteria:
        - Population: All age group patients with influenza like illness.
        - Intervention: Real time RT-PCR based methods/commercial kits
        - Comparator: Centre for Disease Control and Prevention (CDC) protocol as a gold standard.
        - Outcomes: Clinical effectiveness (sensitivity & specificity), diagnostic accuracy and cost effectiveness of real time RT-PCR based methods/commercial kits.
        - Timing: Published between April 01, 2009 to June 21, 2019.
    - **Exclusion criteria:** The studies which were found irrelevant in relation to the research question were excluded. The criteria opted for exclusion is as follows:
        - Rapid Influenza Diagnostic tests (RIDTs), Digital Immuno Assays (DIAs) and Point of care tests (POCs)
        - RT-PCR of other respiratory viruses.
        - Kits/versions not including target for Influenza A (H1N1pdm09).
        - Comparator other than CDC gold standard.
        - Languages other than English.

    The specimen types acceptable for inclusion were nasopharyngeal aspirates, swabs, or washes; nasal aspirates, swabs, or washes; and throat swabs. For a study to be eligible, the index test and comparator needed to test the same clinical specimen. We have finally included the studies demonstrating the research question and PICOS/T strategy, published in English language providing original data on the clinical effectiveness in terms of sensitivity and specificity of real time RT-PCR based

commercial kits for the diagnosis of Influenza A/H1N1pdm09 against CDC based real time RT-PCR method as a gold standard.

4. **Data Extraction:** A data extraction sheet was created in Microsoft Office Excel 2007 and all data pertaining to Author/Journal information, Index test/commercial kit/ kit version, reference test (comparator), patient/clinical data and clinical effectiveness data (overall & specific for H1N1) were extracted. Two reviewers independently extracted data. Disagreements were resolved by consensus or by a third reviewer. Articles that assessed several index tests against a reference standard were counted as several studies; a separate extraction form was completed for each index test. **Table 2** illustrates the characteristics of the studies used for data synthesis & analysis to demonstrate the diagnostic accuracy of real time RT-PCR based kits for the detection of Influenza A H1N1pdm09.
5. **Data Synthesis & Analysis:** For each included study, we have calculated sensitivity, specificity, along with 95% confidence intervals (CIs). All of these parameters have been studied for overall (total no. of viruses detected by the index test) and independently for Influenza A/H1N1pdm09. The pooled overall sensitivities and specificities have also been analyzed irrespective of the index test used. All analysis was carried out using Meta Disc software version 1.4.

Table 1: Search strategy protocol for the identification of studies

Electronic Database	Search strategy used	Filters applied	Articles/ Citations found	Relevant findings
Pubmed	Comparison) OR Influenza) OR H1N1) AND Real time) OR RT PCR) AND CDC AND Sensitivity	Article type: Journal Article, Systematic Reviews, Meta-analysis and Evaluation studies. Text availability: Abstract Publication dates: 01.04.2009 to 21.06.2019 Species: Humans Ages: Birth to 80+ years (all age group) Sort by: Best Match	207	137
PubMed Central (PMC)	Comparison) OR Influenza) OR H1N1) AND Real time) OR RT PCR) AND CDC AND Sensitivity	Article type: Journal Article, Systematic Reviews, Meta-analysis and Evaluation studies. Publication dates: 01.04.2009 to 21.06.2019 Text availability: Abstract	754	2
Wiley Online Library	Comparison) OR Influenza) OR H1N1) AND Real time) OR RT PCR) AND CDC AND Sensitivity	Publication dates: 01.04.2009 to 21.06.2019 Text availability: Abstract	311	0
EMBASE	Comparison) OR Influenza) OR H1N1) AND Real time) OR RT PCR) AND CDC AND Sensitivity	Article type: Journal Article, Systematic Reviews, Meta-analysis and Evaluation studies. Publication dates: 01.04.2009 to 21.06.2019 Text availability: Abstract	71	1
Cochrane Database of Systematic Reviews (CDSR)	Comparison) OR Influenza) OR H1N1) AND Real time) OR RT PCR) AND CDC AND Sensitivity	Article type: Journal Article, Systematic Reviews, Meta-analysis and Evaluation studies. Publication dates: 01.04.2009 to 21.06.2019 Text availability: Abstract	2131	8
CRD-NIHR including DARE, NHS EED, INAHTA-HTA	Comparison) OR Influenza) OR H1N1) AND Real time) OR RT PCR) AND CDC AND Sensitivity	Article type: Journal Article, Systematic Reviews, Meta-analysis and Evaluation studies. Publication dates: 01.04.2009 to 21.06.2019 Text availability: Abstract	545	0
U.S. National Library of Medicine (U.S.NLM)	Comparison) OR Influenza) OR H1N1) AND Real time) OR RT PCR) AND CDC AND Sensitivity	Article type: Journal Article, Systematic Reviews, Meta-analysis and Evaluation studies. Publication dates: 01.04.2009 to 21.06.2019 Text availability: Abstract	235	0

Fig 1: Flowchart of systematic search

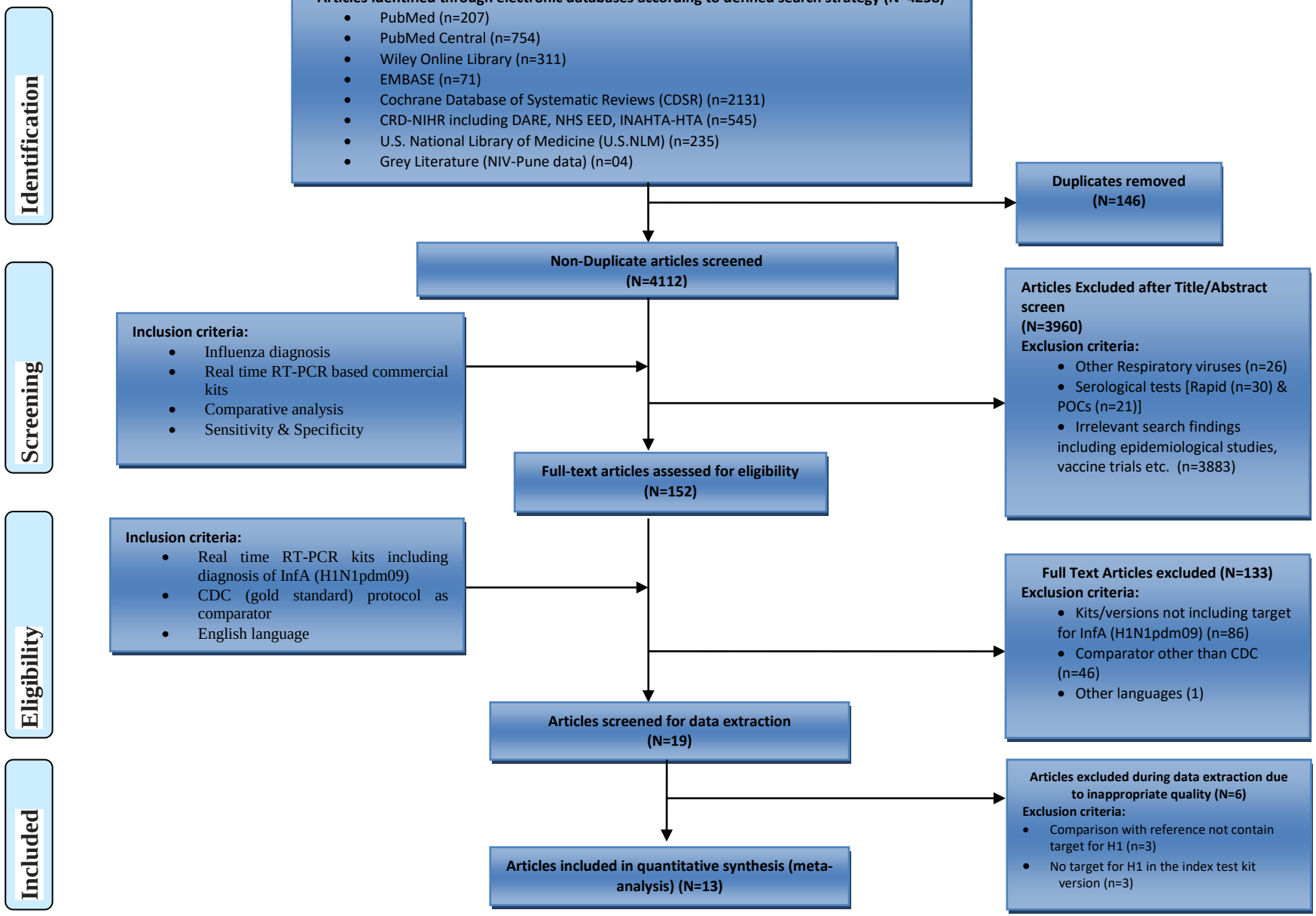


Table 2: Characteristics of the included studies, in all studies CDC /WHO test used as gold standard

Author, year [ref.]	Country	Index Test, kit version, Mfd. country	No. of viruses detected, (Name), [Subtype]	Study type, (Sample type)	Age Group	Sample size	TP	FN	FP	TN
Lalle *et al.* 2011 [1]	Italy	RealStar Influenza Screen & Type RT-PCR kit 5.0 (Astra Diagnostics, Hamburg, Germany)	2, (Inf A & B), [H1N1]	Retrospective, (Nasopharyngeal swab for Inf A and sputum sample for Inf B)	Not Known	81	7	0	24	50
Pierro *et al.* 2013 [2]	Italy	Xpert® Flu assay (Cepheid, USA)	2, (Inf A & B), [H1N1]	Prospective, (Nasopharyngeal swab)	Not Known	60	18	3	2	37
Banerjee 1 *et al.* 2018 [3]	USA	GeneXpert Xpress Flu/RSV (Cepheid,USA)	3(Inf A, Inf B & RSV), [H1N1]	Retrospective, (Nasopharyngeal swabs)	2 months to 80 months (median age=7 months)	225	29	0	0	196
Banerjee 2 *et al.* 2018 [3]	USA	Hologic Panther Fusion Flu A/B/RSV (Fusion)	3(Inf A, Inf B & RSV), [H1N1]	Retrospective, (Nasopharyngeal swabs)	2 months to 80 months (median age=7 months)	225	29	0	0	196
Banerjee 3 *et al.* 2018 [3]	USA	Luminex Aries Flu A/B & RSV (Aries)	3(Inf A, Inf B & RSV), [H1N1]	Retrospective, (Nasopharyngeal swabs)	2 months to 80 months (median age=7 months)	225	28	1	0	196
Banerjee 4 *et al.* 2018 [3]	USA	Influenza A/B & RSV (Liat)	3(Inf A, Inf B & RSV), [H1N1]	Retrospective, (Nasopharyngeal swabs)	2 months to 80 months (median age=7 months)	225	29	0	0	196
Banerjee 5 *et al.* 2018 [3]	USA	Diasorin Simplexa Flu A/B & RSV (Simplexa)	3(Inf A, Inf B & RSV), [H1N1]	Retrospective, (Nasopharyngeal swabs)	2 months to 80 months (median age=7 months)	225	29	0	0	196
Banerjee 6 *et al.* 2018 [3]	USA	Biofire FilmArray Respiratory Panel (RP)	20 (adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, human metapneumovirus, influenza A, influenza A subtype H1, influenza A subtype H3, influenza A subtype 2009 H1, influenza B, parainfluenza virus 1, 2, 3, 4, rhinovirus/ enterovirus, respiratory syncytial virus, Bordetella pertussis, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae.*	Retrospective, (Nasopharyngeal swabs)	2 months to 80 months (median age=7 months)	225	29	0	0	196
Hang *et al.* 2018 [4]	Vietnam	Luminex xTAG Respiratory Viral Panel FAST v2	19 (Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 H1N1, Influenza B, Respiratory Syncytial Virus, Coronavirus NL63, Coronavirus OC43, Coronavirus HKU1, Coronavirus 229E, Parainfluenza 1, 2, 3, 4, Human Metapneumovirus, Enterovirus/Rhinovirus, Adenovirus, and Human Bocavirus)	Retrospective, (Nose & Throat swab)	All Age group (median age of children was 1 year and of adults was 46 years)	442	9	0	1	432

Wenzel *et al.* 2010 [5]	Germany	Roche RealTime Ready Influenza A/H1N1 detection set	1 (Influenza A Matrix gene M2), [H1N1]	Retrospective, (Nasopharyngeal swabs, Nasal wash and nasal swab)	All age group	359	112	2	7	238
Tham *et al.* 2012 [6]	Vietnam	Roche RealTime Ready Influenza A/H1N1 detection set	1 (Influenza A Matrix gene M2), [H1N1]	Retrospective, (Nose & Throat swab)	Age group 1 - 78 years (median age is 16.8 years)	210	105	14	0	91
Selvaraju 1 *et al.* 2010 [7]	USA	Prodesse ProFlu+ multiplex real time RT-PCR Assay (ProFlu+)	4 (Influenza A, Influenza B, RSVA and RSVB), [H1N1]	Retrospective, (Not Known)	children	150	30	0	0	120
Selvaraju 2 *et al.* 2010 [7]	USA	MGB Alert Influenza A/B & RSV RUO assay (MGB)	4 (Influenza A, Influenza B, RSVA and RSVB), [H1N1]	Retrospective, (Not Known)	children	150	26	4	0	120
Barbas *et al.* 2012 [8]	Argentina	Roche RealTime Ready Influenza A/H1N1 detection set	1 (Influenza A Matrix gene M2), [H1N1]	Retrospective, (Nasal or Nasopharyngeal swabs)	Not Known	136	55	17	7	57
Dayakar *et al.* 2018 [9]	India	RealStar Influenza RT-PCR kit 3.0 (Altona Diagnostics, GmbH, Germany)	2, (Inf A & B), [H1N1]	Retrospective, (Nasopharyngeal /throat swabs)	All age group	118	13	0	0	105
In House Report (Xpert Flu, Cepheid)	India	Xpert® Flu assay (Cepheid, France)	2, (Inf A & B), [H1N1]	Retrospective, (Throat/nasal swab)	All age group	149	28	2	0	119
In House Report (TRUPCR)	India	TRUPCR H1N1 detection (IVD) kit	1, (Inf A), [H1N1]	Retrospective, (Throat/nasal swab)	All age group	152	51	3	0	98
In House Report (Artus, Qiagen)	India	Artus Inf A./H1 LC/ RG RT-PCR kit (Qiagen, India Pvt. Ltd.)	2, (Inf A & B), [H1N1]	Retrospective, (Throat/nasal swab)	All age group	151	52	3	1	95
In House Report (Invitrogen)	India	Invitrogen, Superscript III Platinum One Step qRT-PCR kit	1, (Inf A), [H1N1]	Retrospective, (Throat/nasal swab)	All age group	150	43	0	0	107

3.3 Primary Data Collection

Primary data were collected from in-house reports (NIV data), four (n=4) kits evaluated during the financial year 2018-2019.

Validation Study: From four different companies' influenza diagnosis molecular kits [Thermo Fisher Scientific (Invitrogen); TRUPCR H1N1 kit (3BBlackBio Bhopal); Qiagen artus Infl./H1 LC/RG RT-PCR Kit; and Cepheid Xpert® Flu kit] were evalauetd and compared with CDC/WHO gold standard kit.

Primary Costing Study

The bottom-up approach of costing was used, to include prices of various components like consumables, instruments, infrastructure, overheads, Human resource information etc. were collected. For each of the facilities, annualization of capital costs was done. Annual factor was calculated using a discount factor of 3% and the life of the item. A maintenance rate of 10% was applied. Apportioning of joint/shared costs (Personnel, space or equipment that are being used for more than one activity) was calculated. Space cost was calculated by multiplying the estimates of furnished floor size of the facility with the local commercial rental price of the similar space. The total cost of the recurrent resources (consumables) was calculated by multiplying the unit price with the quantity of respective resource consumed. The resources (both capital and recurrent) which were shared in nature and were used in multiple activities were apportioned towards each of the respective activity using appropriate apportioning statistics. Staff members (scientist, technical assistant, technician and multitasking staff) which were jointly involved in a number of activities, proportional time spent in sample processing and testing activities by the staff member was used as an apportioning statistic for allocating their salaries towards these particular activities. Salary for technical assistant, technician and multitasking staff was used for calculation as contractual consolidated salary as per ICMR guidelines.

4. Results

4.1 Results of Systematic Literature Review and Meta-analysis:

1. Electronic database Search Results: A sum total of 4258 records identified through different electronic database searching and 4112 of which were screened after duplicates removal. After applying pre-specified inclusion/exclusion criteria, 3960 articles were found inappropriate and hence excluded after titles and abstracts screening. Rest of the articles (n=152) were selected for full text read and 133 of which were further screened and excluded as not found fit for further inclusions. Nineteen (n=19) studies were finally selected for data extraction and six (n=6) out of which were excluded due to inappropriate/insufficient data provided. We finally included 13 articles (8 Pubmed + 1 EmBase + 4 In-house evaluation reports/Unpublished data) in our meta-analysis of accuracy estimates **(Fig 1)**.

2. Data Extraction: Thirteen (n=13) articles were finally selected for data extraction which includes: [Author/Journal information (title, authors, journal, publication year, country)], [Index test/commercial kit (name of kit, manufacturing company/country, kit version, no. of viruses/subtypes tested, time taken)], [Reference test (CDC)], [Patient/clinical data (sample size, age group, PID, sample type, retrospective/prospective study, time of sample collection etc.)], [Clinical effectiveness data (True positive, True negative, False positive, False negatives)] **[Table 2]**. All of the above mentioned data were extracted from included articles and maintained in Microsoft Office Excel 2007. Data extraction was done independently by two reviewers and the disagreements were resolved by consensus. Two of the included articles (Banerjee *et al*., 2018 & Selvaraju *et al*., 2010) was found to assess several index tests against a reference standard and were counted as several studies and quoted as Banerjee 1, 2, 3……..6 & Selvaraju 1, 2 in this study.

3. Data Synthesis and analysis: All the raw data from included studies were extracted. Further analysis was done using True positives (TP), False negatives (FN), False positives (FP) and True negatives (TN). The overall accuracy of the index test demonstrates the total number and subtypes of viruses detected. Individual accuracy defines specifically for H1N1pdm09. **Diagnostic accuracy of overall kit:** Here we have considered the diagnostic accuracy of the overall kit including different subtypes of the influenza virus. The sensitivity of different kits varied from 46% to 100% whereas the specificity of different kits varied from 87% to 100% among the studies included in the review. Even for the same kit, there was huge variation between different studies both for sensitivity and specificity. (Table 3)

Diagnostic accuracy of H1N1: Here we have considered the diagnostic accuracy of the kit only for H1N1. The sensitivity of different kits varied from 76% to 100% whereas the

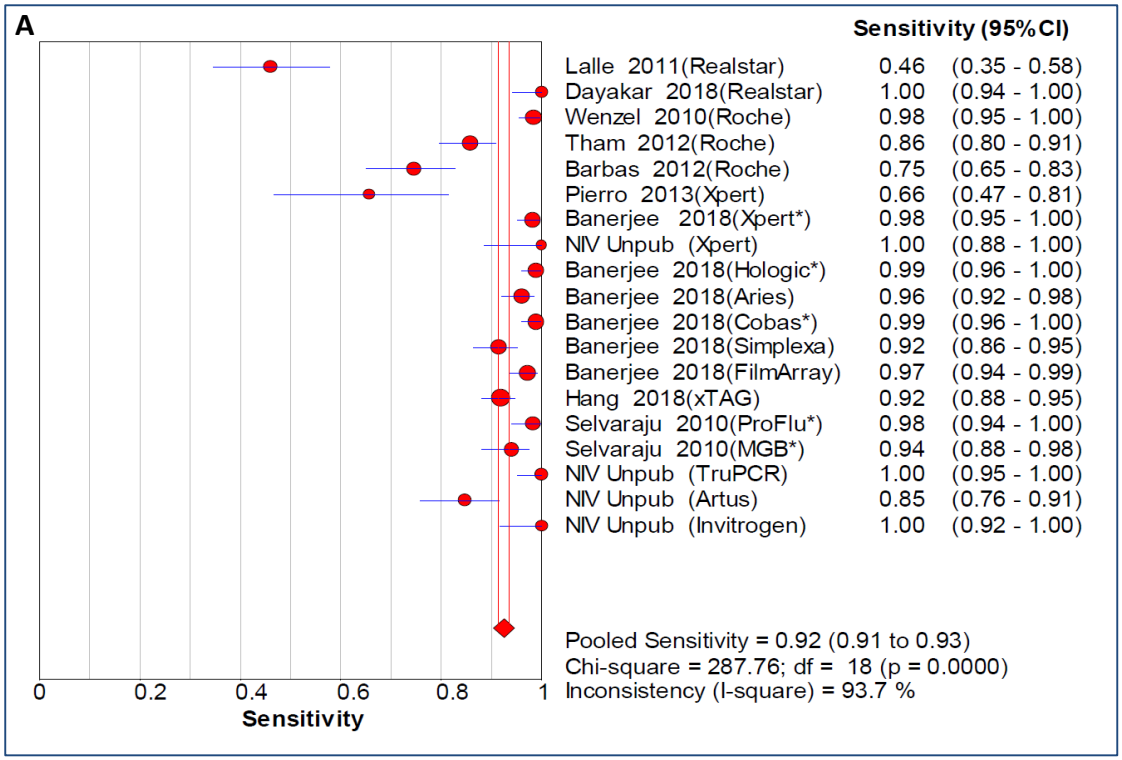
specificity of different kits varied from 67% to 100% among the studies included in the review. Even for the same kit, there was huge variation between different studies both for sensitivity and specificity (Table 4).

Studywise diagnistic results (**Table 3)** and forest plot **(Figure 2)** showed statistically significant heterogeneity. Hence use of pooled estimates will not be appropriate. For H1N1 virus **[Table 4 & Figure 3]** similar results were noted. Therefore, we have used inhouse kit evaluation estimates for comparision purpose and intrepretaion of the results.

Table 3: Diagnostic accuracy of overall kit

Study (Kit name)	Sensitivity	[95% Confidence Interval]		Specificity	[95% Confidence Interval]	
Lalle 2011 (Realstar)	0.461	0.345	0.579	1.000	0.478	1.000
Dayakar 2018 (Realstar)	1.000	0.941	1.000	1.000	0.937	1.000
Wenzel 2010 (Roche)	0.984	0.955	0.997	0.870	0.810	0.917
Tham 2012 (Roche)	0.858	0.796	0.907	0.976	0.871	0.999
Barbas 2012 (Roche)	0.745	0.649	0.826	0.971	0.847	0.999
Pierro 2013 (Xpert Flu)	0.656	0.468	0.814	0.929	0.765	0.991
Banerjee 2018 (Xpress Flu/RSV)	0.983	0.951	0.996	0.938	0.828	0.987
NIV Unpublished (Xpert Flu)	1.000	0.884	1.000	0.992	0.954	1.000
Banerjee 2018 (Hologic)	0.989	0.960	0.999	1.000	0.926	1.000
Banerjee 2018 (Aries)	0.960	0.920	0.984	0.979	0.889	0.999
Banerjee 2018 (Cobas)	0.989	0.960	0.999	0.917	0.800	0.977
Banerjee 2018 (Simplexa)	0.915	0.864	0.952	0.958	0.857	0.995
Banerjee 2018 (FilmArray)	0.972	0.935	0.991	0.917	0.800	0.977
Hang 2018 (xTAG)	0.918	0.881	0.947	0.982	0.979	0.985
Selvaraju 2010 (ProFlu+)	0.983	0.940	0.998	1.000	0.894	1.000
Selvaraju 2010 (MGB*)	0.940	0.881	0.976	1.000	0.894	1.000
NIV Unpublished (TruPCR)	1.000	0.951	1.000	0.949	0.874	0.986
NIV Unpublished (Artus)	0.848	0.758	0.914	0.966	0.883	0.996
NIV Unpublished (Invitrogen)	1.000	0.918	1.000	1.000	0.966	1.000
Pooled	0.924	0.913	0.935	0.978	0.975	0.982
Heterogeneity chi-squared = 287.76 (d.f.= 18) p < 0.001 Inconsistency (I-square) = 93.7 % No. studies = 19.				Heterogeneity chi-squared = 81.27 (d.f.= 18) p < 0.001 Inconsistency (I-square) = 77.9 % No. studies = 19.		

*Not detecting H1 target



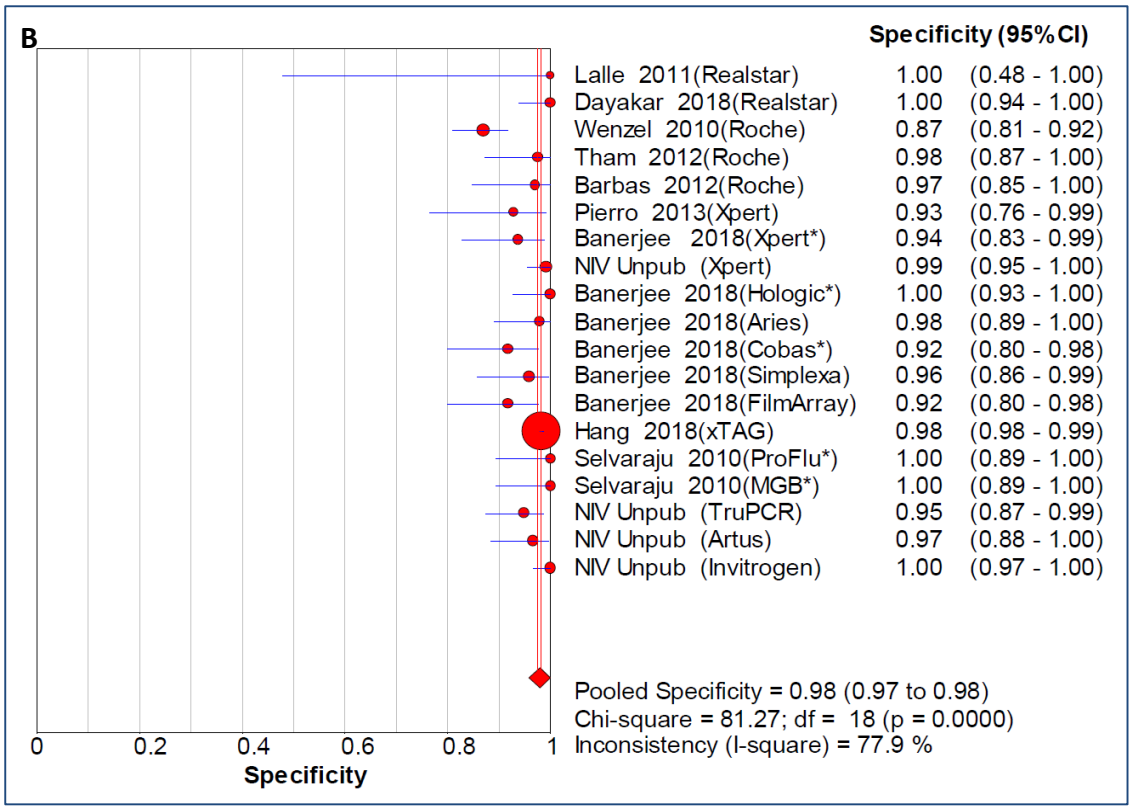


Figure 2: Forest plot for sensitivity (A) and specificity (B) of each individual study for overall kit, the pooled estimate are represented by diamond and the horizontal lines represent 95% confidence intervals (CI).

Table 4: Diagnostic accuracy of H1N1

Study (Kit name)	Sensitivity	[95% Confidence Interval]		Specificity	[95% Confidence Interval]	
Lalle 2011 (Realstar)	1.000	0.590	1.000	0.676	0.557	0.780
Dayakar 2018 (Realstar)	1.000	0.753	1.000	1.000	0.965	1.000
Wenzel 2010 (Roche)	0.982	0.938	0.998	0.971	0.942	0.988
Tham 2012 (Roche)	0.882	0.810	0.934	1.000	0.960	1.000
Barbas 2012 (Roche)	0.764	0.649	0.856	0.891	0.788	0.955
Pierro 2013 (Xpert Flu)	0.857	0.637	0.970	0.949	0.827	0.994
Banerjee 2018 (Xpress Flu/RSV)	1.000	0.881	1.000	1.000	0.981	1.000
NIV Unpublished (Xpert Flu)	0.933	0.779	0.992	1.000	0.969	1.000
Banerjee 2018 (Hologic)	1.000	0.881	1.000	1.000	0.981	1.000
Banerjee 2018 (Aries)	0.966	0.822	0.999	1.000	0.981	1.000
Banerjee 2018 (Cobas)	1.000	0.881	1.000	1.000	0.981	1.000
Banerjee 2018 (Simplexa)	1.000	0.881	1.000	1.000	0.981	1.000
Banerjee 2018 (FilmArray)	1.000	0.881	1.000	1.000	0.981	1.000
Hang 2018 (xTAG)	1.000	0.664	1.000	0.998	0.987	1.000
Selvaraju 2010 (ProFlu+)	1.000	0.884	1.000	1.000	0.970	1.000
Selvaraju 2010 (MGB*)	0.867	0.693	0.962	1.000	0.970	1.000
NIV Unpublished (TruPCR)	0.944	0.846	0.988	1.000	0.963	1.000
NIV Unpublished (Artus)	0.945	0.849	0.989	0.990	0.943	1.000
NIV Unpublished (Invitrogen)	1.000	0.918	1.000	1.000	0.966	1.000
Pooled	0.936	0.917	0.953	0.985	0.980	0.989
Heterogeneity chi-squared = 69.21 (d.f.= 18) p < 0.001 Inconsistency (I-square) = 74.0 % No. studies = 19.				Heterogeneity chi-squared = 196.69 (d.f.= 18) p < 0.001 Inconsistency (I-square) = 90.8 % No. studies = 19.		

*Not detecting H1 target

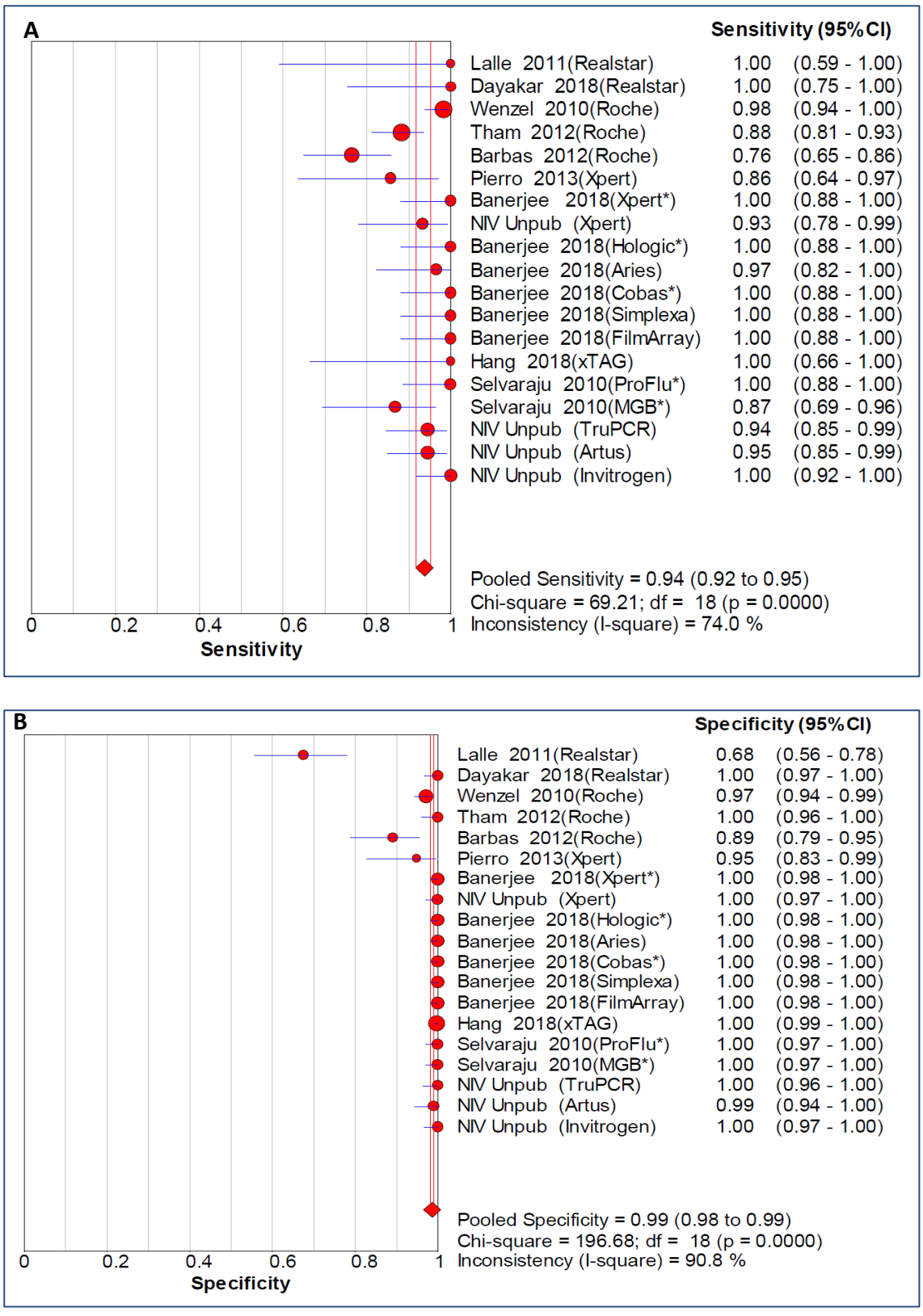


Figure 3: Forest plot for sensitivity (A) and specificity (B) of each individual study for H1N1, the pooled estimate are represented by diamond and the horizontal lines represent 95% confidence intervals (CI).

Primary data Collection

4.2 Validation Study:

On the other hand, data were also collected from in-house reports (NIV data), four (n=4) kits were evaluated during the financial year 2018-2019.

Thermo Fisher Scientific (Invitrogen Pandemic H1N1/09 Assay kit)

Pandemic H1N1/09 Assay Set v3.0 contains components that are Influenza A Assay, Pandemic Influenza A assay, Pandemic H1 Assay and RNase P control Assay. Sensitivity and specificity for Influenza A target of A(H1N1)pdm09 virus was 100% (95% CI: 91.8, 100) and 100% (95% CI: 96.53, 100) respectively. Sensitivity and specificity for Influenza Hemagglutinin H1 target of A/H1N1pdm09 virus was 100% (95% CI: 91.8, 100) and 100% (95% CI: 96.53, 100) respectively. Sensitivity and specificity for Influenza A target of A (H3N2) virus was 100% (95% CI: 85.69, 100) and 100% (95% CI: 97.06, 100) respectively. It takes around 4 hours (which includes sample processing, RNA extraction, real time RT-PCR and result analysis) to complete the test run and 29 samples can be run in one round.

TRUPCR H1N1 kit (3BBlackBio Bhopal)

TRUPCR® H1N1 detection real time RT-PCR kit developed for the detection of Influenza A(H1N1)pdm09 virus in clinical samples. Kit contains master mix, enzyme mix, primer probe mix and positive controls. Kit detects internal control of human source RNaseP, universal Influenza A target, Pandemic (2009) Influenza A target & H1 (hemagglutinin) target of Influenza A(H1N1)pdm09 virus. Sensitivity and specificity for Influenza A target was 100% (95% CI: 95.06, 100) and 94.87% (95% CI: 87.54, 97.99) respectively. Sensitivity and specificity for Influenza H1 target of A(H1N1)pdm09 virus was 94.44% (95% CI: 84.89, 98.09) and 100% (95% CI: 96.23, 100) respectively.

Qiagen artus Infl./H1 LC/RG RT-PCR Kit. For research use only, not for use in diagnostic procedures. The artus Infl./H1 LC/RG RT-PCR Kit constitutes two ready-to-use systems for the detection of influenza A and B viral RNA and novel influenza A (H1N1) viral RNA (2009 H1N1 virus) using reverse transcription–polymerase chain reaction (RT-PCR) on Rotor-Gene Q or LightCycler instruments. The Influenza master contains reagents and enzymes for the specific amplification of influenza virus A genome and influenza virus B genome. **It does not differentiate between Influenza A and B type**. The Influenza H1 master contains reagents and enzymes for the specific amplification of influenza virus H1

(2009 H1N1 virus) genome. Sensitivity and specificity for Influenza target was 84.78% (95% CI: 76.06, 90.71) and 96.61% (95% CI: 88.46, 99.07) respectively. Sensitivity and specificity for Influenza H1 target of A(H1N1)pdm09 virus was 94.55% (95% CI: 85.15, 98.13) and 98.96% (95% CI: 94.33, 99.82) respectively. It takes around 4 hours (which includes sample processing, RNA extraction, real time RT-PCR and result analysis) to complete the test run and 34 or 46 samples can be run in one round depending upon rotor used.

Cepheid Xpert® Flu kit

The Xpert® Flu Assay is an automated in vitro diagnostic test for the qualitative detection and differentiation of **influenza A, influenza B, and influenza A subtype H1N1pdm09** directly from nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens of patients. The assay is performed on Cepheid GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample processing/lysis, purification, nucleic acid amplification, and detection of the viral target in samples using reverse transcriptase real-time PCR assays. Single-use disposable Xpert Flu Assay cartridges that hold the RT-PCR and PCR reagents and perform the detection PCR processes. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. Sensitivity and specificity for FluA target of A(H1N1)09pdm virus tested was 100% (95% CIs: 88.65, 100) and 99% (95% CIs: 95.39, 99.85) respectively. Sensitivity and specificity for influenza hemagglutinin H1 target of A/H1N1pdm09 virus tested was 93.33% (95% CIs: 78.68, 98.15) and 100% (95% CIs: 96.87, 100) respectively. Sensitivity and specificity for Influenza B virus was 96.67% (95% CIs: 83.33, 99.41) and 100% (95% CIs: 96.87, 100) respectively. Sensitivity and specificity for Flu A target of A (H3N2) virus tested was 63% (95% CIs: 38.64, 81.52) and 100% (95% CIs: 97.19, 100) respectively. It takes around 2 hours to complete the test run, 5 minutes of hands on time for sample processing and 1 to 4 samples can be run in one round depending upon the machine module.

The pooled values of sensitivities and specificities established that RT-PCR based diagnosis of H1N1 can be regarded as the gold standard. Among all the four diagnostic kits tested, Invitrogen kit was shown to possess maximum (100%) sensitivity and specificity values followed by Artus (Qiagen) with 94.5% sensitivity and 99% specificity. However, TRUPCR kit was 94.4% sensitive with a specificity of 100%. Sensitivity was least recorded in Xpert (93.3%) while the specificity remains 100%. As per the NIV data reports, we concluded that Invitrogen is the kit with maximum diagnostic effectiveness (100%) and Xpert Flu with least

clinical effectiveness (93.3%). TRUPCR is the indigenous kit (made in Bhopal, India) and reported almost similar sensitivity & specificity in contrast to other imported kits.

4.3 Primary costing study

For costing of kits, we included prices of various components like consumables, instruments, infrastructure, overheads, Human resource information etc. were collected. All the cost and expenditure estimates in the present study were calculated in Indian National Rupees (INR) for the year 2019 **(Table 5)**.

Table 5: Costing of different kits/sample testing

S. No.	Cost Heads for data collection	Invitrogen kit cost/ sample	Qiagen kit cost/ sample	TruPCR kit cost/ sample	Cepheid kit cost/ sample
1	Human Resources	138.84	138.84	138.84	127.59
2	Medical Equipments	99.31	85.02	99.31	112.79
3	Reagents (Medical Consumables)	1675.07	1575.99	1320.1	4060.75
4	Physical Infrastructure	64.35	64.35	64.35	28.35
5	Utilities &Overheads (Water/Electricity/Laundry/ Maintenance etc.)	37.48	37.48	37.48	12.85
6	Total	2015.05	1901.69	1660.08	4342.33

4.4. Final Results:

Literature Review: Diagnostic accuracy of the kits for detection of H1N1 showed huge variation. The sensitivity of different kits varied from 76% to 100% whereas the specificity of different kits varied from 67% to 100% among the studies included in the review. Even for the same kit, there was huge variation between different studies both for sensitivity and specificity. (Table 4)

Validation Study: Invitrogen kit exhibited the highest sensitivity and specificity for detection of H1N1 among 4 kits evaluated in validation study.

Costing Study: Cephied kit was most expnsive with cost/test of Rs.4342, whereas TRUPCR was least expensive with cost/test of Rs. 1660.

Operational Feasibility: Integrated Disease Surveillance Programme (IDSP) assisted lab network of 12 Laboratories are providing laboratory support in terms of testing, providing viral transport medium and diagnostic reagents. The laboratory network of ICMR-VRDL (30 labs) has also been activated to test for H1N1 cases. All these labs are currently using real time RT-PCR test for diagnosis of influenza H1N1 using Invitrogen kit. The present study reconfirms that Invitrogen kit is most cost effective kit for diagnosis of influenza H1N1 with an incremental cost of 355 Rs/test. As the kit uses an open system, it could be used in any real time PCR platform. Already being used in the system, the staff is trained in using the kit also. Therefore, there will be no addional burden in terms of procuring new platforms, or training of staff and cost of kits.

Cost Effectiveness:

- Influenza is an acute event and disease may range from mild symptomts to severe complications depending upon the age and other co-morbidities of the patients. Influenza testing is not needed for all outpatients with signs and symptoms of influenza to make antiviral treatment decisions. If treatment is clinically indicated, antiviral treatment is not withheld from outpatients or hospitalized patients with suspected influenza while awaiting influenza testing results. However, the diagnostic information is valuable for many other reasons. It does have a value of reassurance for the patients and the families and also help them seeking proper care and in taking due cautions.
- If only H1N1 detection (not considering H3N2 and Influenza B) is considered for deciding the cost effectiveness, TRUPCR kit dominated over Qiagen and Cephied kit

with least cost (1660 Rs/test) and highest accuracy (sensitivity 94%, specificity 100%) among these three kits, and thus Qiagen and Cephied were excluded from cost-effectiveness analysis. While comparing the TRUPCR kit and Invitrogen kit, later shows higher accuaray with an incremental cost of 355 Rs/test.

- If accuracy of overall kit is considered including Influenza A and Influenza B, Cephied kit can detect both subtypes in single reaction including the subtypes also. In addition to detecting both subtypes, the Cephied kit can provide results in least of the time. The kit is highly automated and thus easiest to be performed among the four kits. But at the same time, this kit works in a closed system model, that means the kit works only in a given platform and only 1-4 samples can be tested in one go, depending upon the machine module being used.

Table 6: Summary Table

Target*		**Invitrogen Kit**	**Qiagen kit**	**TruPCR kit**	**Cepheid kit**
Cost (Rs) Per Sample		2015	1902	1660	4342
Ease of doing		Easy	Easy	Easy	Easiest
Turn around time		4 hours	4 hours	4 hours	2 hours
Samples in one go		29 Samples	34 or 46 samples, depending upon the rotor used.	29 Samples	1 to 4 samples, depending upon the machine module.
Operational Feasibility		Open system Existing labs equipped with RT-PCR machine	Open system Can be used in existing facilities.	Open system Can be used in existing facilities.	Closed system Health system will need to buy new RT-PCR machines, if this kit is introduced.
Influenza A	Sensitivity (95%CI)	100 (91-100)	84 (76-90)	100 (95-100)	100 (88-100)
	Specificity (95%CI)	100 (96-100)	96 (88-99)	94 (87-97)	99 (95-99)
H1N1	Sensitivity (95%CI)	100 (91-100)	94 (85-98)	94 (84-98)	93 (78-93)
	Specificity	100	98	100	100

	(95%CI)	(96-100)	(94-99)	(96-100)	(96-100)
H3N2	Sensitivity (95%CI) Specificity (95%CI)	100 (85-100) 100 (97-100)			63 (38-81) 100 (97-100)
B	Sensitivity (95%CI) Specificity (95%CI)				96 (83-99) 100 (96-100)

*In-house evaluated kit (Unpublished NIV data as reported in Table 2)

4.5 Study Recommendations:

1. In view of highest diagnostic accuracy (100% sensitivity and 100% specificity) among all the kits evaluated in this study, Invitrogen (CDC/WHO real time RT-PCR) kit is recommended for diagnosis of Category C patients for influenza A/H1N1pdm09 virus from clinical samples with an incremental cost of 355 Rs/test.

2. Diagnostics of H1N1in India, is currently being provided by ICMR-VRDL and NCDC network and all these centres are using real time PCR based technique with Invitrogen kits. The present study reconfirms the fact Invitrogen (CDC/WHO real time RT-PCR) is most cost effective kit for H1N1 diagnostics with no additional burden to the healthcare system.

5. Strengths of the study:

First comprehensive HTA study on realtime PCR kits for H1N1 diagnosis.

- Supported by systematic review.
- Supported by primary study done specifically for this HTA analysis to estimate the diagnostic accuracy of the kits by an in house validation study.
- Supported by primary study done specifically for this HTA analysis to estimate the cost of resources utilized, medical and non-medical cost for H1N1 testing.
- This study also considered highly valuable suggestions and key points that emerged after intensive stakeholder's consultation.

Limitations of the study:

- More kits needs to be evaluated from different companies. Three companies have already approached to NIV for their evaluation. These kits can also be considered in future.

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Annexures 1: **Pandemic H1N1/09 Assay kit- Invitrogen**

Head	Equipment	Unit Price	Quantity	Expected life (yrs) of Equipment	Dicount Rate	Annual Factor	Equivlent Unit Annual Cost Capital	Annual Maintenance Cost	Present Worth value of Maintenance	Annual Cost	Apportioned Cost/ sample	
Capital	Laminar Air Flow	500000	1	10	0.03	0.117230507	58615.2533	5000	3720.469574	62335.72288	12.98660893	
	Benchtop centrifuge with rotor for 2 ml reaction tubes	250000	2	10	0.03	0.117230507	58615.2533	5000	3720.469574	62335.72288	12.98660893	
	7500 Fast Dx Real time PCR instrument (Applied Biosystems) with Laptop & accessories	1800000	1	10	0.03	0.117230507	211014.9119	18000	13393.69047	224408.6024	46.75179216	
	Vertical Autoclave	200000	1	10	0.03	0.117230507	23446.10132	2000	1488.18783	24934.28915	5.194643573	
	Vortex mixer	20000	2	10	0.03	0.117230507	4689.220264	400	297.637566	4986.85783	1.038928715	
	Mini Plate spinner	50000	1	10	0.03	0.117230507	5861.52533	500	372.0469574	6233.572288	1.298660893	
	Mini Spin	20000	1	10	0.03	0.117230507	2344.610132	200	148.818783	2493.428915	0.519464357	
	-20 (freezer)	100000	1	10	0.03	0.117230507	11723.05066	1000	744.0939149	12467.14458	2.597321787	
	Refrigerator	30000	1	10	0.03	0.117230507	3516.915198	300	223.2281745	3740.143373	0.779196536	
	Computer system	40000	1	10	0.03	0.117230507	4689.220264	400	297.637566	4986.85783	1.038928715	
	UPS	100000	1	10	0.03	0.117230507	11723.05066	1000	744.0939149	12467.14458	2.597321787	
	Air conditioner (AC)	40000	4	10	0.03	0.117230507	18756.88106	1600	1190.550264	19947.43132	4.155714858	
	Printer cum scanner	15000	1	5	0.03	0.218354571	3275.318571	300	258.7826353	3534.101206	0.736271085	
	Pipettes: (Genaxy)											
	2-20ul (NPX-20)	15000	3	5	0.03	0.218354571	9825.955713	900	776.3479059	10602.30362	2.208813254	
	20-200ul (NPX-200)	15000	3	5	0.03	0.218354571	9825.955713	900	776.3479059	10602.30362	2.208813254	
	100-1000ul	15000	3	5	0.03	0.218354	9825.9	900	776.34	10602.	2.2088	

	(NPX-1000)					571	55713		79059	30362	13254	
	Total Amount per sample	99.30790209										
Manpower	Designation	salary	Number of persons	hours work each day	number of days work per month	Nu. Of hours per month	Salary per hour	No. of hours for this work/ day	no. of hours for spent each month	Number of specimens processed per month	Amount spent per month for staff salary	Amount spent for required staff per sample
	Techcician	18000	1	8	22	176	102.2727273	2	44	400	4500	11.25
	Research Assistant	31000	2	8	22	176	176.1363636	4	176	400	31000	77.5
	Scientist	126300	1	8	22	176	717.6136364	1	22	400	15787.5	39.46875
	Multitasking staff	17000	1	8	22	176	96.59090909	2	44	400	4250	10.625
	Total Amount per sample	138.84375										
Physical Infrastructure	Room	Service for which room is used	Area (in sqft)	Monthly Rental Price (per sqft), space used for other purpose also	Rental to be used for calculation/month	Rental to be used for calculation/sample						
	Separation Room	Sample separation	78	7020	7020	17.55						
	RNA extraction room	RNA extraction	160	14400	7200	18						
	Clean room	Reagent preparation	160	14400	7200	18						
	Machine room	PCR	192	17280	4320	10.8						

	Total Amount per sample	64.35										
Overhead	Cost Head	Consumption (specific to service in cost)/day	Frequency of use	Consumption (specific to service in cost)/ Months	Cost/test							
	Electricity	408.835	Every day	12265.05	30.662625							
	Water	36	Every day	1080	2.7							
	Telephone/ Fax/Printers	10	Every day	300	0.75							
	Laundry	10	Every day	300	0.75							
	Sanitation	10	Every day	300	0.75							
	Sterilization	20	Every day	600	1.5							
	Others	5	Every day	150	0.375							
	Total Amount per sample	37.487625										
Reagents/ Consumables	Total	1675.07										
Grand total (Rs)	2015.059277											

Annexures 2: Artus- Qiagen

Capital	Equipment	Unit Price	Quantity	Expected life (yrs) of Equipment	Dicount Rate	Annual Factor	Equivlent Unit Annual Cost Capital	Annual Maintenance Cost	Present Worth value of Maintenance	Annual Cost	Apportioned Cost/ sample	
	Laminar Air Flow	500000	1	10	0.03	0.117230507	58615.2533	5000	3720.469574	62335.72288	12.98660893	
	Benchtop centrifuge with rotor for 2 ml reaction tubes	250000	2	10	0.03	0.117230507	58615.2533	5000	3720.469574	62335.72288	12.98660893	
	Rotor-Gene® Q 5plex HRM platform, cat no. 9001580	1250000	1	10	0.03	0.117230507	146538.1333	12500	9301.173936	155839.3072	32.46652233	
	Vertical Autoclave	200000	1	10	0.03	0.117230507	23446.10132	2000	1488.18783	24934.28915	5.194643573	
	Vortex mixer	20000	2	10	0.03	0.117230507	4689.220264	400	297.637566	4986.85783	1.038928715	
	Mini Plate spinner	50000	1	10	0.03	0.117230507	5861.52533	500	372.0469574	6233.572288	1.298660893	
	Mini Spin	20000	1	10	0.03	0.117230507	2344.610132	200	148.818783	2493.428915	0.519464357	
	-20 (freezer)	100000	1	10	0.03	0.117230507	11723.05066	1000	744.0939149	12467.14458	2.597321787	
	Refrigerator	30000	1	10	0.03	0.117230507	3516.915198	300	223.2281745	3740.143373	0.779196536	
	Computer system	40000	1	10	0.03	0.117230507	4689.220264	400	297.637566	4986.85783	1.038928715	
	UPS	100000	1	10	0.03	0.117230507	11723.05066	1000	744.0939149	12467.14458	2.597321787	
	Air conditioner (AC)	40000	4	10	0.03	0.117230507	18756.88106	1600	1190.550264	19947.43132	4.155714858	
	Printer cum scanner	15000	1	5	0.03	0.218354571	3275.318571	300	258.7826353	3534.101206	0.736271085	
	Pipettes: (Genaxy)											
	2-20ul (NPX-20)	15000	3	5	0.03	0.218354571	9825.955713	900	776.3479059	10602.30362	2.208813254	
	20-200ul (NPX-200)	15000	3	5	0.03	0.218354571	9825.955713	900	776.3479059	10602.30362	2.208813254	
	100-1000ul (NPX-1000)	15000	3	5	0.03	0.218354571	9825.955713	900	776.3479059	10602.30362	2.208813254	
	Total Amount per sample	85.02263226										
Manpower	Designation	salary	Number	hours work	number of days	Nu. Of hours per	Salary per	No. of hours	no. of hours	Number of	Amount	Amount

			of persons	each day	work per month	month	hour	for this work/ day	for spent each month	specimens processed per month	spent per month for staff salary	spent for required staff per sample
	Techcician	18000	1	8	22	176	102.2727273	2	44	400	4500	11.25
	Research Assistant	31000	2	8	22	176	176.1363636	4	176	400	31000	77.5
	Scientist	126300	1	8	22	176	717.6136364	1	22	400	15787.5	39.46875
	Multitasking staff	17000	1	8	22	176	96.59090909	2	44	400	4250	10.625
	Total Amount per sample	138.8475										
Physical Infrastructure	Room	Service for which room is used	Area (in sqft)	Monthly Rental Price (per sqft), space used for other purpose also	Rental to be used for calculation/month	Rental calculation/sample						
	Separation Room	Sample separation	78	7020	7020	17.55						
	RNA extraction room	RNA extraction	160	14400	7200	18						
	Clean room	Reagent preparation	160	14400	7200	18						
	Machine room	PCR	192	17280	4320	10.8						
	Total Amount per sample	64.35										
Overhead	Cost Head	Consumption (specific to service in cost)/day	Frequency of use	Consumption (specific to service in cost)/ Month	Cost/test							

				s								
	Electricity	408.835	Every day	12265.05	30.662625							
	Water	36	Every day	1080	2.7							
	Telephone/Fax/Printers	10	Every day	300	0.75							
	Laundry	10	Every day	300	0.75							
	Sanitation	10	Every day	300	0.75							
	Sterilization	20	Every day	600	1.5							
	Others	5	Every day	150	0.375							
	Total Amount per sample	37.487625										
Reagents/ Consumables	Total	1575.99										
Grand total (Rs)	1901.694007											

Annexures 3: TRUPCR - 3B BlackBio Bhopal

Capital	Equipment	Unit Price	Quantity	Expected life (yrs) of Equipment	Dicount Rate	Annual Factor	Equivlent Unit Annual Cost Capital	Annual Maintenance Cost	Present Worth value of Maintenance	Annual Cost	Apportioned Cost/ sample	
	Laminar Air Flow	500000	1	10	0.03	0.117230507	58615.2533	5000	3720.469574	62335.72288	12.98660893	
	Benchtop centrifuge with rotor for 2 ml reaction tubes	250000	2	10	0.03	0.117230507	58615.2533	5000	3720.469574	62335.72288	12.98660893	
	7500 Fast Dx Real time PCR instrument (Applied Biosystems) with Laptop & accessories	1800000	1	10	0.03	0.117230507	211014.9119	18000	13393.69047	224408.6024	46.75179216	
	Vertical Autoclave	200000	1	10	0.03	0.117230507	23446.10132	2000	1488.18783	24934.28915	5.194643573	
	Vortex mixer	20000	2	10	0.03	0.117230507	4689.220264	400	297.637566	4986.85783	1.038928715	
	Mini Plate spinner	50000	1	10	0.03	0.117230507	5861.52533	500	372.0469574	6233.572288	1.298660893	
	Mini Spin	20000	1	10	0.03	0.117230507	2344.610132	200	148.818783	2493.428915	0.519464357	
	-20 (freezer)	100000	1	10	0.03	0.117230507	11723.05066	1000	744.0939149	12467.14458	2.597321787	
	Refrigerator	30000	1	10	0.03	0.117230507	3516.915198	300	223.2281745	3740.143373	0.779196536	
	Computer system	40000	1	10	0.03	0.117230507	4689.220264	400	297.637566	4986.85783	1.038928715	
	UPS	100000	1	10	0.03	0.117230507	11723.05066	1000	744.0939149	12467.14458	2.597321787	
	Air conditioner (AC)	40000	4	10	0.03	0.117230507	18756.88106	1600	1190.550264	19947.43132	4.155714858	
	Printer cum scanner	15000	1	5	0.03	0.218354571	3275.318571	300	258.7826353	3534.101206	0.736271085	
	Pipettes: (Genaxy)											
	2-20ul (NPX-20)	15000	3	5	0.03	0.218354571	9825.955713	900	776.3479059	10602.30362	2.208813254	
	20-200ul (NPX-200)	15000	3	5	0.03	0.218354571	9825.955713	900	776.3479059	10602.30362	2.208813254	
	100-1000ul	15000	3	5	0.03	0.218354	9825.9	900	776.34	10602.	2.2088	

	(NPX-1000)					571	55713		79059	30362	13254	
	Total Amount per sample	99.30790209										
Manpower	Designation	salary	Number of persons	hours work each day	number of days work per month	Nu. Of hours per month	Salary per hour	No. of hours for this work/ day	no. of hours for spent each month	Number of specimens processed per month	Amount spent per month for staff salary	Amount spent for required staff per sample
	Techcician	18000	1	8	22	176	102.2727273	2	44	400	4500	11.25
	Research Assistant	31000	2	8	22	176	176.1363636	4	176	400	31000	77.5
	Scientist	126300	1	8	22	176	717.6136364	1	22	400	15787.5	39.46875
	Multitasking staff	17000	1	8	22	176	96.59090909	2	44	400	4250	10.625
	Total Amount per sample	138.84375										
Physical Infrastructure	Room	Service for which room is used	Area (in sqft)	Monthly Rental Price (per sqft), space used for other purpose also	Rental to be used for calculation/month	Rental to be used for calculation/sample						
	Separation Room	Sample separation	78	7020	7020	17.55						
	RNA extraction room	RNA extraction	160	14400	7200	18						
	Clean room	Reagent preparation	160	14400	7200	18						
	Machine room	PCR	192	17280	4320	10.8						
	Total	64.35										

	Amount per sample											
Overhead	Cost Head	Consumption (specific to service in cost)/day	Frequency of use	Consumption (specific to service in cost)/ Months	Cost/test							
	Electricity	408.835	Every day	12265.05	30.662625							
	Water	36	Every day	1080	2.7							
	Telephone/ Fax/Printers	10	Every day	300	0.75							
	Laundry	10	Every day	300	0.75							
	Sanitation	10	Every day	300	0.75							
	Sterilization	20	Every day	600	1.5							
	Others	5	Every day	150	0.375							
	Total Amount per sample	37.487625										
Reagents/ Consumables		1320.1										
Grand total (Rs)	1660.089277											

Annexures 4: Xpert Flu- Cepheid

Capital	Equipment	Unit Price	Quantity	Expected life (yrs) of Equipment	Dicount Rate	Annual Factor	Equivlent Unit Annual Cost Capital	Annual Maintenance Cost	Present Worth value of Maintenance	Annual Cost	Apportioned Cost/ sample	
	Laminar Air Flow	500000	1	10	0.03	0.117230507	58615.2533	5000	3720.469574	62335.72288	12.98660893	
	Benchtop centrifuge with rotor for 2 ml reaction tubes	250000	1	10	0.03	0.117230507	29307.62665	2500	1860.234787	31167.86144	6.493304466	
	GeneXpert GXIV-4-L (4modules)	3009000	1	10	0.03	0.117230507	352746.5944	30090	22389.7859	375136.3803	78.15341256	
	Vertical Autoclave	200000	1	10	0.03	0.117230507	23446.10132	2000	1488.18783	24934.28915	5.194643573	
	Vortex mixer	20000	1	10	0.03	0.117230507	2344.610132	200	148.818783	2493.428915	0.519464357	
	Refrigerator	30000	1	10	0.03	0.117230507	3516.915198	300	223.2281745	3740.143373	0.779196536	
	Computer system	40000	1	10	0.03	0.117230507	4689.220264	400	297.637566	4986.85783	1.038928715	
	UPS	100000	1	10	0.03	0.117230507	11723.05066	1000	744.0939149	12467.14458	2.597321787	
	Air conditioner (AC)	40000	2	10	0.03	0.117230507	9378.440528	800	595.2751319	9973.71566	2.077857429	
	Printer cum scanner	15000	1	5	0.03	0.218354571	3275.318571	300	258.7826353	3534.101206	0.736271085	
	Pipettes: (Genaxy)											
	2-20ul (NPX-20)	15000	1	5	0.03	0.218354571	3275.318571	300	258.7826353	3534.101206	0.736271085	
	20-200ul (NPX-200)	15000	1	5	0.03	0.218354571	3275.318571	300	258.7826353	3534.101206	0.736271085	
	100-1000ul (NPX-1000)	15000	1	5	0.03	0.218354571	3275.318571	300	258.7826353	3534.101206	0.736271085	
	Total Amount per sample	112.7858227										
Manpower	Designation	salary	Number of persons	hours work each day	number of days work per month	Nu. Of hours per month	Salary per hour	No. of hours for this work/ day	no. of hours for spent each month	Number of specimens processed	Amount spent per month for	Amount spent for required

										per month	staff salary	staff per sample
	Techcician	18000	0	8	22	176	102.2727273	2	0	400	0	0
	Research Assistant	31000	1	8	22	176	176.1363636	8	176	400	31000	77.5
	Scientist	126300	1	8	22	176	717.6136364	1	22	400	15787.5	39.46875
	Multitasking staff	17000	1	8	22	176	96.59090909	2	44	400	4250	10.625
	Total Amount per sample	127.59375										
Physical Infrastructure	Room	Service for which room is used	Area (in sqft)	Monthly Rental Price (per sqft), space used for other purpose also	Rental to be used for calculation/month	Rental to be used for calculation/sample						
	Separation Room	Sample separation	78	7020	7020	17.55						
	Machine room	PCR	192	17280	4320	10.8						
	Total Amount per sample	28.35										
Overhead	Cost Head	Consumption (specific to service in cost)/day	Frequency of use	Consumption (specific to service in cost)/Months	Cost/test							
	Electricity	98.35	Every day	2950.5	7.37625							
	Water	18	Every day	540	1.35							
	Telephone/ Fax/Printers	10	Every day	300	0.75							
	Laundry	10	Every	300	0.75							

			day									
	Sanitation	10	Every day	300	0.75							
	Sterilization	20	Every day	600	1.5							
	Others	5	Every day	150	0.375							
	Total Amount per sample	12.85125										
Reagents/ Consumables		4060.75										
Grand total (Rs)	4342.330823											