ORIGINAL ARTICLE

Analysis of diagnostic parameters of Truenat HPV for detecting Human papilloma virus: A study from a tertiary care hospital

Hemant G Deshpande[1], Prachi V Prasad[2], Manasi V Chavan[2], Charusheela R Gore[3]
Chandrashekhar G Raut[2]*
[1]Department of Obstetrics & Gynaecology, [2]Interdisciplinary Research, Central Research Facility,
[3]Department of Pathology, Dr. D.Y. Patil Medical College, Hospital and Research Centre,
Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune-411018 (Maharashtra) India

Abstract

Background: Human Papillomavirus (HPV) is a major etiological factor in cervical cancer, making rapid and accurate detection crucial for diagnosis. Prolonged processing times, high costs, and advanced infrastructures are the drawbacks of conventional diagnostic techniques. *Aim and Objectives:* To compare the diagnostic performance of the portable, chip-based Truenat HPV-HR kit with TRUPCR HPV real-time polymerase chain reaction (RT-PCR) and Pap smear tests. *Material and Methods:* Cervical swabs were obtained from women aged 20-69 years for a comparative diagnostic study for the detection of HPV. The diagnostic parameters were computed using SPSS version 29.0.2.0 and Microsoft Excel. Concordance between the methods was calculated using Cohen's kappa coefficient. *Results:* HPV was detected in 12.07% using TRUPCR HPV and 5.17% using Truenat HPV-HR. Truenat showed a sensitivity and specificity of 42.86% and 100% for 16/31 and 18/45 HPV genotypes, respectively. Concordance between the methods was 93.1% ($\kappa = 0.57$), indicating moderate agreement. HPV prevalence was highest among individuals aged 31–40 years (42.86%) and in women with normal cytology (15.38%). *Conclusion:* The Truenat HPV-HR test showed significant concurrence with TRUPCR HPV, although no association with cytological results was found. This suggests that incorporating PCR could potentially improve early diagnosis, bridge diagnostic gaps, and enhance patient outcomes.

Keywords: Human papillomavirus, Pap smear, TRUPCR, Real-time PCR, Truenat

Introduction

Cervical cancer is the fourth most common malignancy globally and continues to be a major health concern for women [1]. Notably, India accounts for 9.0% of cervical cancer incidences and 8.7% of deaths [2]. The most prevalent viral infection of the reproductive system, Human Papillomavirus (HPV) is a significant risk factor causing over 99% of cervical, 90% of anal, 65% of vaginal, 50% of vulvar, and 45-90% of oropharyngeal cancers [3]. These are further categorized into fifteen high-risk - 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82 three potentially high-risk – 66, 53, 26, and several low-risk HPV types- CP6108, 81, 72, 70, 61, 54, 44, 43, 42, 40, 11, 6 [4]. In response to this public health challenge, the World Health Organization (WHO) urged global action in 2018 to eliminate cervical cancer by 2030, establishing 90-70-90 targets: 90% HPV vaccination coverage for girls by age 15, 70% of women screened by 35 and 45, 90% treatment for precancer, and 90% management for invasive cancer [5].

The adoption of Pap smear screening programs has proven effective in many countries; however, developing countries still face 80% of the world-wide burden [6]. In regions like rural India, limited access to healthcare leads to under-diagnosis of cervical cancer, while HPV prevalence remains elevated, underscoring the need for intensified efforts in these areas [7]. Additionally, a significant concern arises from the detection of HPV in women with normal cytology. The development of invasive cervical cancer results from persistent high-risk HPV (HR-HPV) infections without early cytological signs [8, 9]. Therefore, identifying at-risk individuals through HPV testing enables earlier intervention, appropriate monitoring, and supports vaccination strategies.

Consequently, there has been a shift towards alternative approaches such as molecular HR-HPV tests and Point-of-Care Testing (POCT) devices, offering wider access to diagnosis with greater sensitivity and specificity. WHO guidelines now recommend HPV DNA screening every 5-10 years for women aged 30 and above, reflecting this evolving approach [8]. The 2018 guidelines by Federation of Obstetric and Gynecological Societies of India recommend HPV DNA testing in good resource settings [10].

Real-time Polymerase Chain Reaction (RT-PCR) is the gold standard for HPV diagnosis, but its cost and complexity pose challenges for widespread implementation, particularly in resource-constrained settings. Hence, there is a growing demand for rapid, affordable molecular diagnostic tests with comparable accuracy. The Truelab® device (Molbio Diagnostics, Goa, India) is commercially available for diagnosing over 25 diseases, including rabies, tuberculosis, hepatitis B, dengue, H1N1 influenza, chikungunya, SARS-CoV-2 and malaria. Truenat HPV-HR is a semi-quantitative detection performed using a microchip that carries test and batch-related information including standard values for quantitation. It uses TaqMan probe-based RT-PCR technology to detect four HR-HPV types: 16, 31, 18, and 45. In light of these advancements, our objective was to estimate the diagnostic parameters of the Truenat® HPV-HR kit in detecting HR-HPV by comparing it with the in vitro diagnostics approved TRUPCR® HPV High Risk genotyping kit (Kilpest, India Ltd., Bhopal) and Pap smear results.

Material and Methods

A comparative diagnostic study was conducted between January 2023 and August 2023, at the Molecular Diagnostics Laboratory of Dr. D. Y. Patil Medical College, Hospital, and Research Centre, Pimpri, Pune, India. A total of 58 cervical swab samples were collected from sexually active or married women aged 20-69 years who were undergoing routine HPV testing or cervical cancer screening as part of their clinical assessment. Information regarding demographic details, sexual and reproductive history was acquired from the laboratory request form. Ethical clearance was granted from the Institutional Ethics Sub-Committee with reference number I.E.S.C./W/74/2024. Written informed consent was obtained from all participants prior to collection.

For Truenat HPV-HR detection, samples were collected from the transformation zone using the nylon flocculated cervical swab, transferred into Trueprep AUTO transport media, whereas for RT-PCR swabs were collected in viral transport media

(Himedia, India) and transported to the laboratory. Collected samples were stored at 2-8°C till further processing.

HPV detection using Truenat HPV-HR

The Truelab work setup comprises a Trueprep AUTO sample processing device and a Truelab Duo real-time quantitative micro-PCR analyzer. Nucleic acid extraction was performed using Trueprep AUTO v2 Universal Cartridge-based sample prep kit. Briefly, 500 µL cervical samples pretreated with lysis buffer were added to the cartridge chamber and placed in the device. The automated process produced purified DNA within 20 minutes, which was then collected from the elute chamber. This DNA was analysed using real-time PCR on the Truelab Duo micro-PCR analyzer, which differentiates four HR-HPV genotypes: 16/31 and 18/45. The test was initiated by selecting the profile and entering sample details. Six micro-liters of DNA were added to a centrifuge tube containing lyophilized PCR master mix, then transferred to a microchip well. The system auto-interprets results after 40 minutes, interpreting "Not Detected" if amplification is observed in the Internal Positive Control (IPC) but not in channels 16/31 and 18/45, and "Detected" if amplification is observed in channels 16/31, 18/45 or both.

HPV detection using TRUPCR HPV-HR with 16/18 genotyping kit

This real-time amplification assay enables qualitative detection and genotyping of 14 HPV strains: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. DNA was extracted from 500 µL of sample using the TRUPCR tissue DNA extraction kit (Kilpest, India Ltd., Bhopal) as per the manufacturer's instructions. The kit contains a mastermix, primer probe mix-1,2,3,4,5, positive and negative controls. For each sample, 10 µL of the corresponding primer-probe mix and 10 µL of mastermix were combined to prepare a 20 µL reaction mix, to which 10 µL of extracted DNA was added. Thermal cycling was performed on the Quant Studio 12K Flex RT-PCR system (Applied Biosystems, US) at 94˚C for 10 minutes, followed by 40 cycles of 94˚C for 15 seconds and 60˚C for one minute. The sigmoid shape of curves with a threshold cutoff below 35 was considered positive, and above 35 as negative.

Cytology

After removing any obscuring mucus, cervical cells were collected and smeared onto a glass slide using an Ayres spatula and endocervical brushes. The slide was fixed in 95% ethanol and sent to Pathology for cytological analysis. Cellular changes were classified as Negative for Intraepithelial Lesion or Malignancy (NILM), Atypical Squamous Cells of Undetermined Significance (AS-CUS), Atypical Glandular Cells of Undetermined Significance (AGUS), Low-Grade Intraepithelial Lesion (LSIL), High-Grade Squamous Intraepithelial Lesions (HSIL) or malignancy. The workflow of the study is depicted in Figure 1.

Statistical analysis

Descriptive statistical analysis was performed using standard metrics. Categorical variables such as test positivity, cytological categories, and genotype distributions were presented as frequencies (n) and percentages (%). Continuous variables, such as age, were summarized using mean ± Standard Deviation (SD). Diagnostic test performance was evaluated using measures of sensitivity, specificity, Positive Predictive Value (PPV), Negative Predic-

tive Value (NPV), and overall diagnostic accuracy with 95% confidence intervals. Cohen's kappa (κ) was used to assess agreement between the two diagnostic methods. Statistical analysis were conducted using Microsoft Excel and IBM Statistical Package for Social Sciences (SPSS, Chicago, US) 29.0.2.0. A value of $p < 0.05$ was considered statistically significant.

Results

A total of 58 cervical samples were tested, of which 12.07% tested positive by TRUPCR HPV, and 5.17% by Truenat HPV-HR. All three Truenat-positive samples were also positive by RT-PCR, resulting in no false-positive results for Truenat. However, it missed four samples (6.9%) that were positive by RT-PCR. Among the 51 samples negative by TRUPCR HPV, Truenat accurately detected all as negative. No samples were positive with Truenat but negative with TRUPCR HPV. The overall positivity rate observed with TRUPCR HPV was 12.07%.

The mean age of the patients was 37.8 ± 9.4 years. Among the seven positive samples by TRUPCR HPV, the highest prevalence of HR-HPV was observed in three samples (42.86%) from the 31–40 age group followed by two (28.57%) from the 41–50 age group, and one each (14.3%) from the 21–30 and 51–60 age groups. Of the three samples positive by Truenat, two (66.7%) were from the 31–40 age group and one (33.3%) from the 41–50 age group. No HR-HPV cases were detected in individuals over 60 years. These results suggest a higher likelihood of HR-HPV infection in the middle-aged population (31–50 years).

TRUPCR HPV detected multiple high-risk HPV genotypes, with HPV 56 identified in two samples, and HPV 68, HPV 52, HPV 66, HPV 33, HPV 31, HPV 58, HPV 18, and HPV 16 appearing either individually or as part of co-infections in other samples as shown in Figure 2. Coinfection was detected in three (42.86%) of the TRUPCR HPV positive cases. However, Truenat HPV-HR, which targets only genotypes 16, 18, 31, and 45, detected fewer cases, with no evidence of co-infections.

Cytological analysis data were available for 45 samples, with no malignancies identified. Inflammatory changes were observed in 66.67% (n = 30) of smears, while 28.89% (n = 13) were categorized as NILM. ASC-US was detected in two (4.44%) cases. Among the seven positive cases identified by TRUPCR HPV, two (28.57%) had NILM, and three (42.86%) showed inflammatory changes. Of the three positive cases detected by Truenat, one was NILM, one exhibited inflammatory cytology, and data for one case was unavailable. Additionally, one patient with ASC-US tested positive for HPV. Table 1 shows the comparative test data of both the test kits and Pap smear results. No significant association was observed between Pap smear findings and TRUPCR HPV or Truenat HPV whereas a statistically significant association was observed between TRUPCR HPV or Truenat HPV results.

The Truenat HPV-HR test showed comparable diagnostic parameters to the gold standard RT-PCR, with an overall diagnostic accuracy of 93.10%. The sensitivity, specificity, PPV, and NPV values are mentioned in Table 2. Notably, when analysing only samples containing genotypes targeted by Truenat HPV-HR (16, 18, 31, and 45), all performance metrics, including sensitivity, specificity, PPV, and NPV, were 100%. Also, the concordance between the methods was 93.1% (κ = 0.57), indicating moderate agreement.

Table 1: Comparing test results of Truenat HPV-HR, TRUPCR and Pap smear

Test	NILM	Inflammatory + ASCUS	Total	*p*
Truenat Positive	1	1	2	
Truenat Negative	12	31	43	0.778 NS
Total	13	32	45	
TRUPCR Positive	2	4	6	
TRUPCR Negative	11	28	39	0.264 NS
Total	13	32	45	
Test	**RT-PCR Positive**	**RT-PCR Negative**		
Truenat Positive	03	00	03	
Truenat Negative	04	51	55	0.0011*
Total	07	51	58	

*NS- Not significant, *- significant at p<0.05*

Table 2: Statistical data of diagnostic parameters of Truenat HPV-HR test

Diagnostic parameters	Value	95% Confidence Interval
Sensitivity	42.86%	15.82 % - 74.95%
Specificity	100%	92.99% - 100%
PPV	100%	43.85% - 100%
NPV	92.73%	82.74% - 97.14%
Diagnostic accuracy	93.10%	86.6% - 99.6%

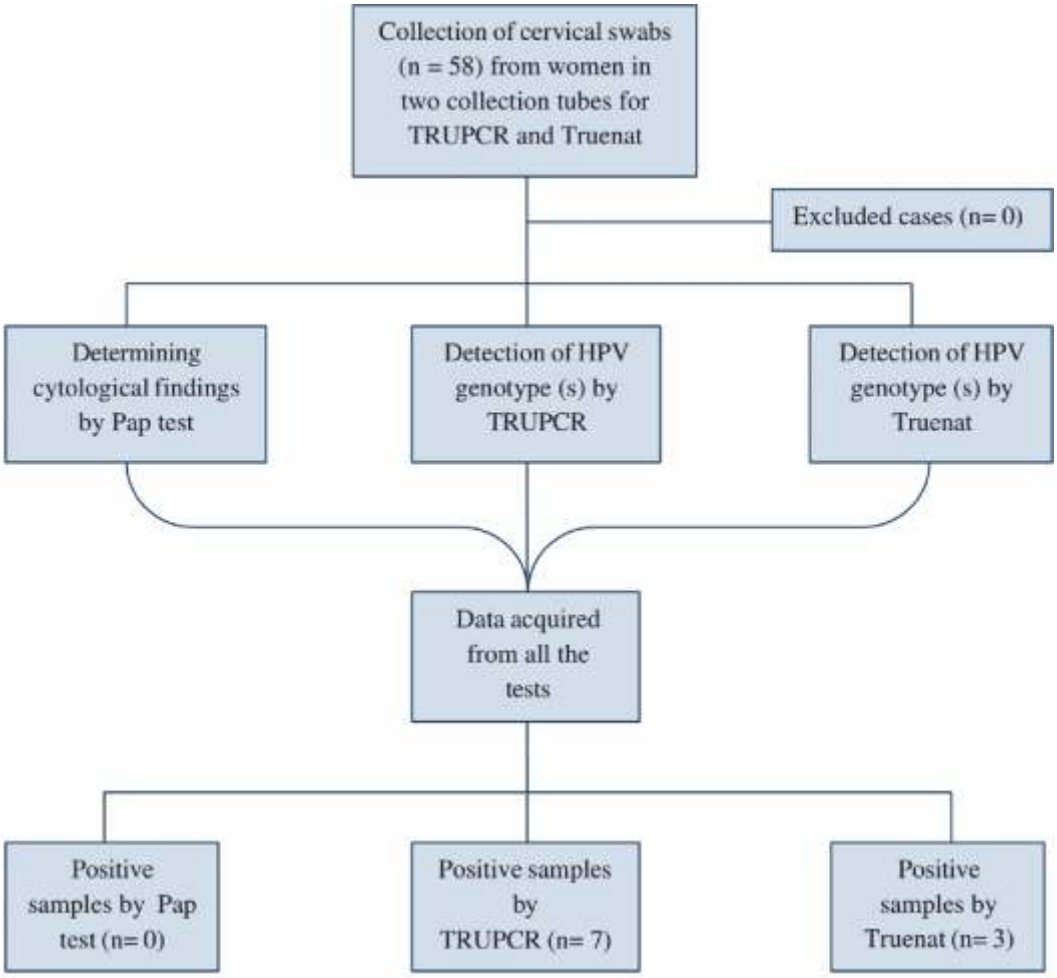

Figure 1: Flow chart illustrating the order of steps involved in the comparative assessment

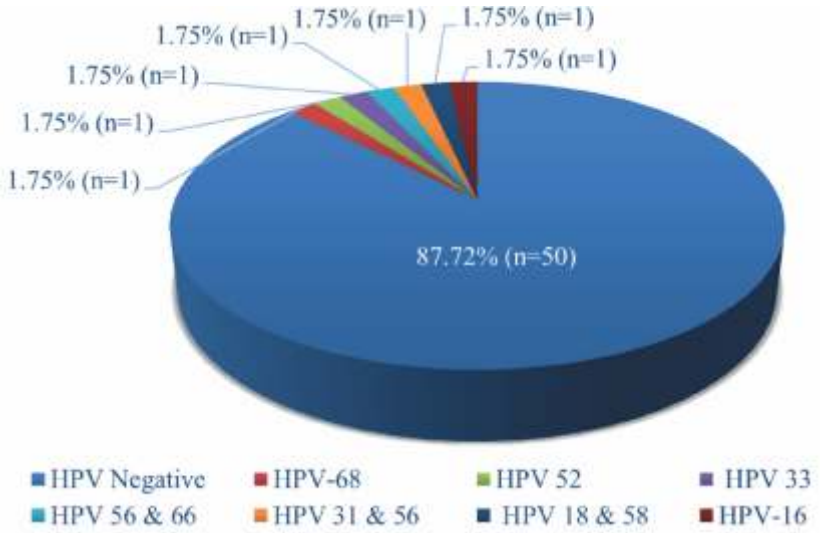

Figure 2: Genotype-wise distribution of HPV detected using RT-PCR

Discussion

Cervical cancer screening is crucial for the early detection of precancerous lesions. The death rate for cervical cancer among participating women has significantly decreased by 41–92% in developed nations because of the successful implementation of screening programs [8]. In relevance to this, the present study aimed to assess the diagnostic accuracy and clinical applicability of the Truenat® HPV-HR test in comparison to TRUPCR HPV RT-PCR, the gold standard, for detecting HR-HPV in cervical samples.

The overall HR-HPV prevalence in our study was 12.07%, aligning with Ethiopian data (12.9%) but significantly lower than rates reported in certain Indian states, such as Andhra Pradesh (87.8%) and Chennai (99.4%) [11-13]. Our findings suggest a high rate of HPV positivity in the 31-50 age group, which is consistent with previous studies indicating a higher risk of HPV infection among women aged 25 to 34 years [14]. No HR-HPV cases were detected in individuals over 60 years, possibly reflecting reduced exposure risk in older populations. However, there have been reports of an increased HPV prevalence in women aged ≥ 50 years [15]. The most common HPV genotype observed in our study was HPV56, accounting for 28.6% cases. In concordance with the results from previous studies [16], we found that 57.14% of cases had a single HPV genotype infection, while 42.86% had a coinfection.

Although no samples tested through conventional Pap smear findings showed signs of malignancy, a significant percentage (66.67%) displayed inflammation. If left untreated, persistent inflammation can increase the likelihood of developing cervical lesions, as observed in certain studies [17]. ASCUS was detected in 4.44% of the women screened, slightly greater than earlier reports which found ASCUS in one [18]. In women with normal cytology, 15.38% tested positive for single genotypes — HPV 16 and HPV 68, while co-infection was not detected. However, among those with inflammatory cytology, 6.7% were infected with single HPV genotypes— 33 and 52, and 3.3% had co-infections. This finding aligns with studies from New Delhi [19] and reinforces that individuals with negative cytology but positive for high-risk HPV types should have frequent check-ups, since they may be at a higher risk to acquire cancer. Furthermore, when comparing the Truenat HPV-HR and TRUPCR HPV results with conventional cytology, no significant association was found. This finding aligns with previous studies reported from New Delhi [19].

In a recent study, Hariprasadb *et al.* evaluated the Truenat HPV-HR for HPV detection in cervical specimens, reporting high sensitivity (97.7%) and specificity (98.9%) alongside strong PPV (87.8%) and NPV (99.8%) [20]. However, our findings showed a slightly lower sensitivity for Truenat (42.86%), attributed to its limited genotypic range. Despite this, its specificity and PPV remained exceptionally high at 100%, NPV (92.73%) and overall diagnostic accuracy (93.10%) were comparable to previous findings.

The chip-based technology of Truenat HPV-HR facilitates rapid and real-time results, making it suitable for screening in primary healthcare centres and remote locations. It is portable, cost-effective, and user-friendly, enhancing its applicability for resource-limited settings where prompt HR-HPV detection is critical. However, it does not provide detailed genotyping information. The TRUPCR

HPV High Risk genotyping kit, in contrast, identifies 14 distinct HR-HPV genotypes that may be essential for epidemiological studies and personalized treatment plans. Nevertheless, its reliance on specialized training and infrastructure makes it more suited for high-resource settings. However, to make HPV testing accessible in developing countries, it is essential to reduce the cost and simplify the testing process. Real-time PCR testing allows processing multiple samples simultaneously but is time-consuming. Whereas Truenat systems can run only one cartridge for RNA extraction and two samples concurrently for PCR in a Duo unit within an hour. Nevertheless, Truenat could ease the load on small testing labs and enhance the overall diagnostics.

A few limitations of the assessment include the small sample size (n = 58), which limits the general applicability, necessitating larger population-based studies to validate the diagnostic performance of Truenat HPV-HR across diverse populations and clinical contexts.

Conclusion

These findings indicate that relying solely on Pap smears may not be sufficient, as HPV was detected using PCR in patients with normal cytology. A significant concurrence between the TRUPCR HPV and Truenat HPV-HR test was observed, indicating it to be an effective method for the early detection of HPV before cytological changes appear. The Truenat HPV-HR test demonstrates high specificity and diagnostic accuracy for its target genotypes, making it a valuable tool for HPV screening in resource-limited settings.

Acknowledgements

We would like to acknowledge Suresh Babu TV, Shaithilya AN from Bigtec Labs, Bengaluru. We are grateful to Mr. Shrishail Rodagikar for logistics support, Ms. Pragati Deshmukh and Mr. Ajay Koli for being involved in the detection of the Human papillomavirus from clinical samples.

References

1. Abdulghani MM, VahyaAQ, Hilmi MN, Hussain AF. Study of CD31 IHC expression in dysplastic and malignant lesions of the cervix and its correlation to IHC expression of HPV (16E6 + 18E6). *J Krishna Inst Med Sci Univ* 2024; 13(2):127-35.
2. International Agency for Research on cancer. World Health Organization (WHO). Global Cancer Observatory. Globocan 2022. (2024). Accessed: August 10, 2024. Available from: https://gco.iarc.who.int/media/globocan/factsheets/populations/356-india-fact-sheet.pdf
3. Petca A, Borislavschi A, Zvanca M, Petca RC, Sandru F, Dumitrascu M. Non-sexual HPV transmission and role of vaccination for a better future (Review). *Exp Ther Med* 2020; 20(6):186.
4. Coorevits L, Traen A, Bingé L, Van Dorpe J, Praet M, Boelens J, *et al*. Are vaginal swabs comparable to cervical smears for human papillomavirus DNA testing? *J Gynecol Oncol* 2018; 29(1):e8.
5. World Health Organization (WHO). Cervical Cancer Elimination Initiative. Global. Accessed: August 14, 2024. Available from: https://www.who.int/initiatives/cervical-cancer-elimination-initiative
6. Gravitt PE, Paul P, Katki HA, Vendantham H, Ramakrishna G, Sudula M, *et al*. CATCH Study Team. Effectiveness of VIA, Pap, and HPV DNA testing in a cervical cancer screening program in a peri-urban community in Andhra Pradesh, India. *PLoS One* 2010; 5(10):e13711.

7. Joshi S, Sankaranarayanan R. Opportunities for cervical cancer prevention in India. *J Krishna Inst Med Sci Univ* 2015; 4(1): 8-17.
8. Huber J, Mueller A, Sailer M, Regidor PA. Human papillomavirus persistence or clearance after infection in reproductive age. What is the status? Review of the literature and new data of a vaginal gel containing silicate dioxide, citric acid, and selenite. *Womens Health (Lond)* 2021;17:17455065211020702.
9. Patil SS. Cervical cancer prevention in the era of HPV vaccination: The road ahead. *J Krishna Inst Med Sci Univ* 2024; 13(4): 1-2.
10. Bhatla N, Singhal S, Saraiya U, Srivastava S, Bhalerao S, Shamsunder S, *et al.* (on behalf of FOGSI Expert group). Screening and management of preinvasive lesions of the cervix: good clinical practice recommendations from the Federation of Obstetrics and Gynaecologic Societies of India (FOGSI). *J Obstet Gynaecol Res* 2020; 46(2):201–14.
11. Deksissa ZM, Tesfamichael FA, Ferede HA. Prevalence and factors associated with VIA positive result among clients screened at Family Guidance Association of Ethiopia, south west area office, Jimma model clinic, Jimma, Ethiopia 2013: a cross-sectional study. *BMC Res Notes* 2015; 8:618.
12. Sowjanya AP, Jain M, Poli UR, Padma S, Das M, Shah KV, *et al.* Prevalence and distribution of high-risk human Papilloma virus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh, India. *BMC Infect Dis* 2005; 5:116.
13. Franceschi S, Rajkumar T, Vaccarella S, Gajalakshmi V, Sharmila A, Snijders PJ, *et al.* Human Papillomavirus and risk factors for cervical cancer in Chennai, India: a case-control study. *Int J Cancer* 2003; 107(1):127-33.
14. Dutta S, Begum R, Mazumder Indra D, Mandal SS, Mondal R, Biswas J, *et al.* Prevalence of human papillomavirus in women without cervical cancer: a population-based study in Eastern India. *Int J Gynecol Pathol* 2012; 31(2):178-83.
15. Sarma U, Mahanta J, Borkakoty BJ, Talukdar KL. Detection of Human Papilloma Virus DNA from dry paper cervical smear-a hospital based study. *Int J Life Sci Med Res* 2013; 3:82-7.
16. Gupta S, Purwar S, Gupta P, Halder A, Gupta A, Pushpalatha K, John JH. Burden and associated genotype patterns of high-risk human papilloma virus infection and cervical cytology abnormalities among women in Central India. *Infect Dis Obstet Gynecol* 2022; 18; 2022:3932110.
17. Bhutia K, Puri M, Gami N, Aggarwal K, Trivedi SS. Persistent inflammation on Pap smear: Does it warrant evaluation? *Indian J Cancer* 2011; 48(2):220–2.
18. Verma A, Verma S, Vashist S, Attri S, Singhal A. A study on cervical cancer screening in symptomatic women using Pap smear in a tertiary care hospital in rural area of Himachal Pradesh, India. *Middle East Fertil Soc J* 2017; 22:39–42.
19. Arora R, Kumar A, Prusty BK, Kailash U, Batra S, Das BC. Prevalence of high-risk human papillomavirus (HR-HPV) types 16 and 18 in healthy women with cytologically negative Pap smear. *Eur J Obstet Gynecol Reprod Biol* 2005; 121(1):104-9.
20. Hariprasad R, Tulsyan S, Babu R, Dhanasekaran K, Thakur N, Hussain S, *et al.* Evaluation of a chip-based, point-of-care, portable, real-time micro PCR analyzer for the detection of high-risk human papillomavirus in uterine cervix in India. *JCO Glob Oncol* 2020; 6:1147-54.

***Author for Correspondence:**

Dr. Chandrashekhar G. Raut, Interdisciplinary Research, Central Research Facility, Dr. D.Y. Patil Medical College, Hospital and Research Centre, Dr. D.Y. Patil Vidyapeeth, Pimpri, Pune-411018

Email: cgrpn2810@gmail.com Cell:8999079968

How to cite this article:

Deshpande HG, Prasad PV, Chavan MV, Gore CR, Raut CG. Analysis of diagnostic parameters of Truenat HPV for detecting human papilloma virus: A study from a tertiary care hospital. *J Krishna Inst Med Sci Univ* 2025; 14(1):57-65.

***Submitted:** 11-Oct-2024 **Accepted:** 15-Dec-2024 **Published:** 01-Jan-2025*