Cost Effective Salivary Viral DNA Isolation in Limited Resource Setting – A Pilot study

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Abstract-

Background: The various diagnostic procedures that requires isolation of viral products from serum, saliva and other body fluids involves cumbersome extraction kits. These kits are very expensive and involve highly technique sensitive procedures. Aim: This pilot study involves systematic review and exploring an option of utilizing the available laboratory materials to perform viral DNA isolation and ensuring the best quality of the isolated DNA through the agar-gel electrophoresis followed by image recording. Materials and methods: Deep throat gargle followed by vigorous rinsing of oral cavity done after mild scrapping of posterior tongue and anterior faucial pillars using ice cream stick in order to ensure enough squamous cell collection. The collected Oral rinse was stored in -80 till the DNA isolation procedure.The isolated DNA was captured through Agar-gel electrophoresis and imaged through special analyzer. Conclusion: Equivalent quality of DNA as well as RNA were isolated through this comparativelycost effective method.

INTRODUCTION:

The various diagnostic procedures that requires isolation of viral products from serum, saliva and other body fluids involves cumbersome extraction kits. These kits are very expensive[1] and involve highly technique sensitive procedures. Manual DNA extractions cost very less and can be carried out using basic laboratory reagents and still provide good quality DNA materials for genomic studies, HLA typing as well as viral product identification[2]. This pilot study involves literature review and exploring an option of utilizing the available laboratory materials to perform viral DNA isolation and ensuring the best quality of the isolated DNA through the agarose-gel electrophoresis followed by image recording.

MATERIALS AND METHODS:

The basic literature search wascarried out in order to obtain the details regarding the available information on the studies which have utilized the kits as well as oral rinse as a protocol for DNA extraction. Search was based on PubMed and the search criteria utilized included the following.

SEARCH CRITERIA:

HPV[All Fields] AND ("manuals as topic"[MeSH Terms] OR ("manuals"[All Fields] AND "topic"[All Fields]) OR "manuals as topic"[All Fields] OR "manual"[All Fields]) AND ("dna"[MeSH Terms] OR "dna"[All Fields]) AND ("isolation and purification"[Subheading] OR ("isolation"[All Fields] AND "purification"[All Fields]) OR "isolation and purification"[All Fields] OR "isolation"[All Fields]) AND ("mouthwashes"[All Fields] OR "mouthwashes"[MeSH Terms] OR "mouthwashes"[All Fields] OR ("oral"[All Fields] AND "rinse"[All Fields]) OR "oral rinse"[All Fields])

A total of 43 articles were obtained after excluding the other articles based on search criteria. The Oral rinse were used along with DNA extraction kits [Broutian TR et al. (2010), Kreimer AR et al. (2012), Deshmukh KL et al. (2017)] for High risk HPV detection in three of the studies whereas manual methods tried were nil.

Eight persons who conformed to the inclusion criteria were selected and asked to do a deep throat gargle using 7ml Normal saline solution (NS 500, Fresenius Kabi, Goa) after scrapping the posterior tongue and anterior faucial pillars using a sterile icecream stick. The obtained oral rinse was subjected to centrifugation and subsequent DNA extractions. The obtained DNA were run in an Agaorse gel electrophoresis and the obtained gel was subjected to UV light illumination as well as

PROCEDURES:

1. Oral Rinse Collection:
    i. Informed consent was obtained from patients who comply with the inclusion criteria of the study
    ii. Patient made to sit in a comfortable position after washing their mouth in plain water to remove food debris.
    iii. A 10ml falcon tube containing 7ml sterile normal saline solution was given to them and instructed to do a deep throat gargling procedure.
    iv. A blunt ice cream stick was taken and scrapped the posterior tongue and anterior faucial pillars in a mild action without causing discomfort or gag reflex.
    v. To the collected oral rinse 100µL of proteinase K (Himedia, India) was added and stored in -80 (Subzero, India) till the DNA isolation procedure.

2. DNA Extraction Method:

    1. The collected saliva was centrifuged at 13000rpm for 10minutes
    2. Discard the Supernatant of the centrifugate
    3. To the pellet add 500 µl of lysis buffer
    4. Incubate at 65°C for 90minutes with occasional tapping of the tube.
    5. Cool sample at room temperature
    6. Add 5M NaCl (250µl) to each tube and vortex at high speed.
    7. Keep at 4°C for 10 minutes
    8. Centrifuge at 10000RPM for 5minutes
    9. Pipette out 600µl of supernatant without disturbing the pellet
    10. Add 600µl of isopropanol. Invert gently and centrifuge at 10000RPM for 5minutes.
    11. Discard supernatant carefully without dislodging the pellet
    12. Add 300µl of 70% ethanol.
    13. Centrifuge at high speed for 5minutes
    14. Discard supernatant carefully without dislodging the pellet.
    15. Airdry overnight
    16. Add 100µl of TE buffer or nuclease free water.

Agarose gel electrophoresis (Orange, India) was carried out to visualize the DNA obtained. The obtained gel block was recorded using UV light transilluminator (Orange, India) as well as Gelstan documentation system (Medicare, India).

RESULTS:The extent of DNA obtained from the method as portrayed in figure 3 was of good quantity. The method is also cost effective (Table 1) as observed from the comparative cost per samples between the available kits and manual methods.

DISCUSSION: The DNA extraction kits involve cumbersome procedures which are technique sensitive and expensive[2,3]. The results obtained demonstrate that the customized methods obtained by combining the basic laboratory chemicals still provides sufficient quantity of DNA (Figure 5). The proteinase K usage also can be

determined depending on the purpose for which the oral rinse need to be collected[4,5]. The total cost involved is only one tenth of that cost of an extraction kit (Table 1). The only disadvantage may be the storage time of extracted DNA before PCR analysis which needs immediate processing as the yield tends to go down on long term storage[2].

CONCLUSION: Techniques using kits required a more cumbersome procedure which were technique sensitive and expensive. The methods mentioned in this study are relatively simple and can be carried out with the available materials in genetics lab and would be useful in a low resource setting. Equivalent quality of DNA was isolated through this comparatively cost-effective method.

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Figure 1: Literature search results based on customized manual methods of DNA extraction from Oral Rinse.

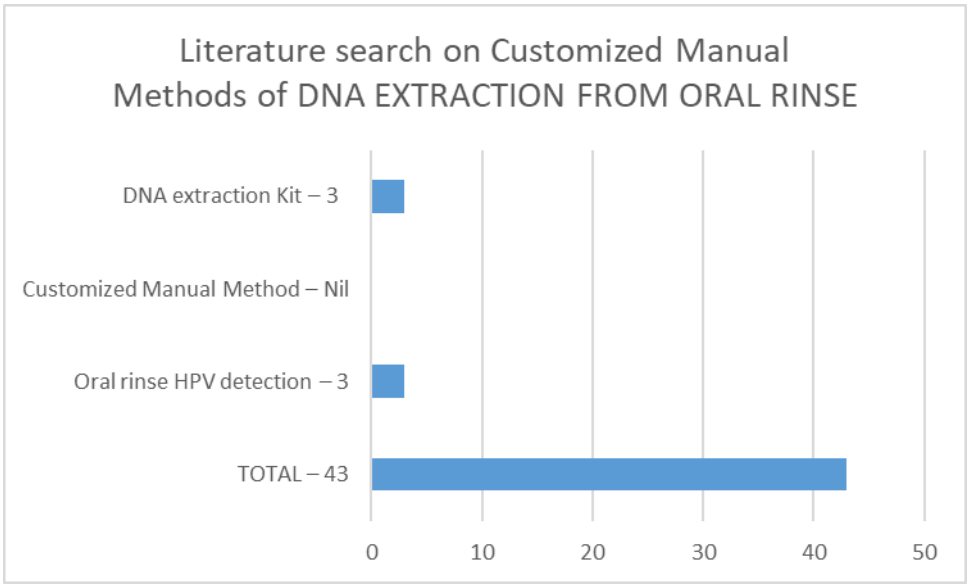

Figure 2: Literature search results based on total sample tested in the selected three studies.

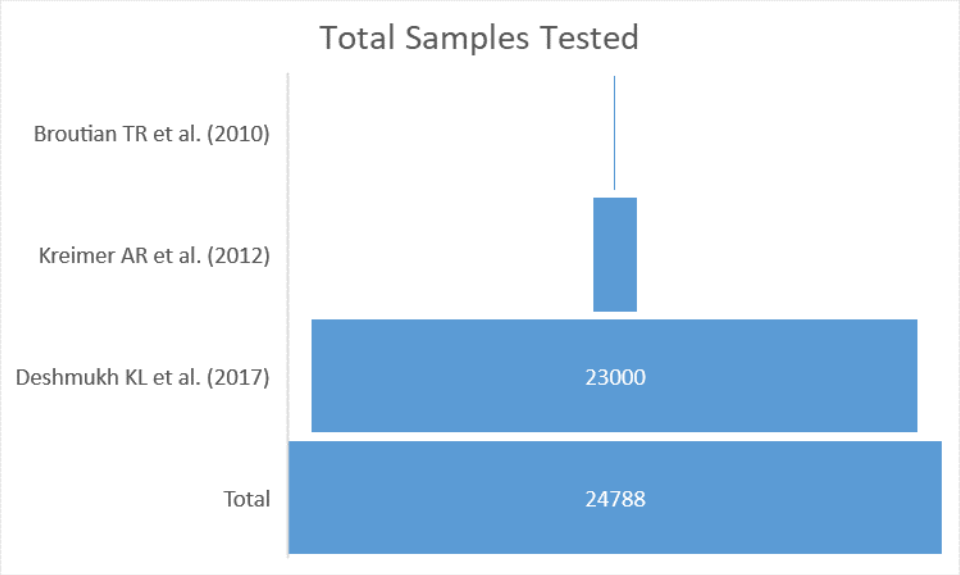

Figure 3: DNA visualization - Gelstan

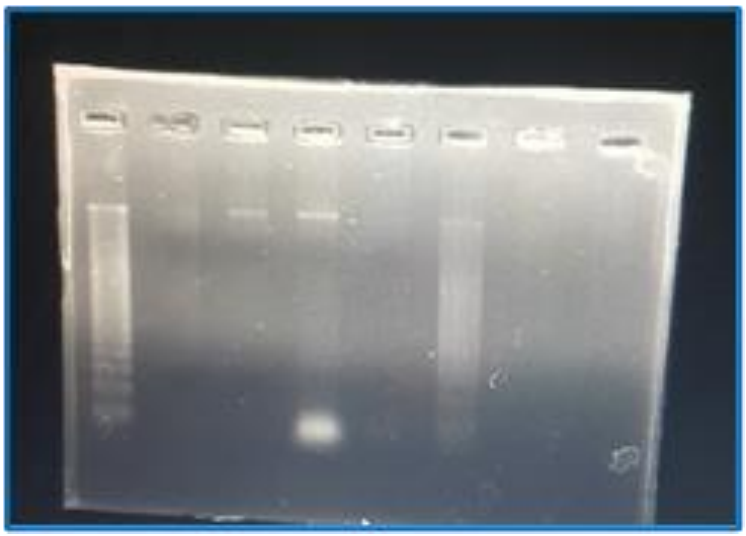

Table 1: The cost comparison between manual methods and that using kits

SL. NO	Methods	Cost per sample in rupees
1.	DNA extraction kits	390-585
2.	Customized methods with proteinase K	98-104
3.	Customized methods without proteinase K	52-58