

NP Screen® Assay Risk Assessment for Nasopharyngeal Carcinoma (NPC)

Caution: This device is restricted to sales by or on the order of a physician.

Intended Use:

NP Screen® assay is an *in-vitro* deoxyribonucleic acid (DNA) assay intended for the semi-quantitative detection of the Epstein-Barr virus (EBV) in specimen collected from the posterior nasopharyngeal epithelium.

Indication for Use:

NP Screen® assay is indicated for use as a routine ambulatory screen to aid in the diagnosis of Epstein-Barr virus (EBV) associated undifferentiated nasopharyngeal carcinoma (NPC) in males and females of Chinese ethnicity over 20 years of age (high-risk patients). In the absence of other clinical findings, patients with normal **NP Screen®** assay results are considered to be at a low contemporary risk for NPC. Patients with abnormal **NP Screen®** assay results should be evaluated for evidence of NPC according to standard of practice in oncology.

NP Screen® assay is intended for use as a screen only, formal confirmatory nasopharyngeal biopsy and histopathologic analysis is required for diagnosis of cancer. Diagnosis of cancer shall not be solely based on **NP Screen®** assay results.

Background

NP Screen® assay provides information about the EBV DNA status in specimen derived from the posterior nasopharynx of a high-risk patient. The **NP Screen®** assay result is compared to a reference standard to determine the patient's risk for NPC.

The annual mortality for nasopharyngeal cancer exceeds 50,000 worldwide [1] and within both endemic and sporadic incidence populations the most common malignant tumor of the nasopharynx is nasopharyngeal carcinoma (NPC) [2-5, ACS¹]. The WHO classifies NPC into three histologic subtypes: Type I, keratinizing squamous cell carcinoma (well differentiated); Type II, nonkeratinizing carcinoma (differentiated) and Type III, undifferentiated carcinoma [6]. Type III, NPC accounts for 95% of nasopharyngeal cancers in high-incidence populations and most of the remaining 5% is associated with Type II, NPC [4,7]. Type I, NPC is predominant in low-incidence populations [8].

NPC occurs sporadically in the United States, Canada and Europe (<1/100,000), generally representing 0.2% of all malignancies. NPC is one of the most common cancers affecting Hong Kong Chinese and is highly prevalent in the southern part of China (20-100/100,000), intermediate incidence rates are prevalent in Southeast Asia, Mediterranean rim countries, coastal regions of Africa and in the indigenous populations (Inuit) of the northern reaches of Alaska, Canada and Greenland (10-40/100,000). Emigrants relocating from endemic areas to non endemic areas such as United States and Canada maintain their elevated risk status while first and second generations carry progressively lower risk. NPC has a peak incidence in the 50 to 54 age range, with incidence starting to climb steadily to this point after 20 years of age. Males are affected more frequently than females, 2:1 [9-12].

NPC arises from the nasopharyngeal epithelium, usually in the fossa of Rosenmüller located at the lateral aspects of the posterior nasopharynx. The tumor tends to spread submucosally and remains clinically inconspicuous during the early stages of the disease and patients can

¹ American Cancer Society, Cancer Reference Information for NPC (2006)

present with a neck mass or other head and neck symptoms with no clinically identifiable abnormality in the nasopharynx [13,14]. Ten year survival rates for tumor stages I, II, III and IV are reported to be 77%, 65%, 54% and 29% respectively indicating better outcomes through early diagnosis and treatment [15].

Epstein-Barr virus (EBV) is a causative agent, or an early event co-factor, in the pathogenesis of NPC and the tumor and its metastases are clonal proliferations from the original EBV infected progenitor [26,28,29,56,57]. Within the virion the EBV genome exists as a 172kbp linear double stranded DNA molecule [51]. After infection and entry into the cell the EBV genome circularizes to produce a nonintegrated intracellular latent episomal form of the virus. The infected cell harbors multiple episomal copies (from a few to over fifty copies) which replicate with cell division [26-29]. Evidence of EBV episomes and EBV coded gene products are detected in virtually all preinvasive lesions, primary and metastatic tumors, for both endemic and sporadic population cases [24,25], regardless of histologic subtype or degree of differentiation or geographic origin of the patient [16-23].

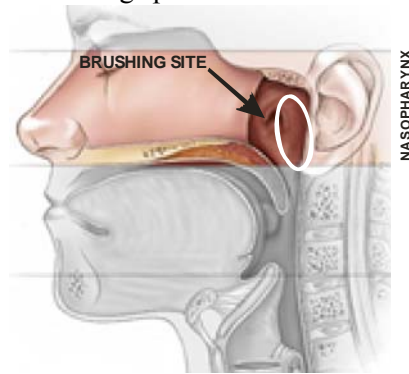
Most humans are infected with EBV by the age of 20 years and serologic markers of this event are detectable for the life of the host. At any given time 20% of the infected population will experience an asymptomatic, transient reactivation with heightened antibodies for EBV antigens [33,34]. In high-risk populations, persistent, reactivated EBV infection is antecedent to the evolution of NPC and in southern China population based screening has validated the use of the EBV serology for the preclinical detection of NPC [34-40,48], however only a relatively few seropositive patients will actually develop NPC [41], which reduces the utility of EBV serology for prediction of NPC risk [49,50].

Given the ubiquitous nature of EBV and its association with NPC, the utility of EBV as a diagnostic marker for NPC is dependant on the virus's distribution in the healthy patient especially in or on the nasopharyngeal mucosa. Clinical studies [30-32,43-47], have demonstrated the absence of EBV in or on the posterior nasopharyngeal mucosa in healthy patients or the epithelium adjacent to NPC lesions in disease patients while other clinical studies [42] have shown the potential for a more specific NPC screening methodology by using PCR to detect EBV levels in specimen collected, trans-nasally, directly from within the boundaries of the anatomic origin for the NPC primary reporting good correlation between detected EBV levels and clinical diagnosis with a clinical sensitivity and specificity of, 90% and 99%, respectively using a high-risk cohort of, $n=157$, healthy patients and, $n=21$, diagnosed cases of NPC. Recent PCR studies using the **NP Screen®** trans-oral brushing method, where the collection of specimen is constrained to the anatomic origin of the NPC primary (Figure 1), showed a high correlation between EBV detection levels and NPC diagnosis reporting clinical sensitivity and specificity of, 98.7% and 99.6%, respectively with a high-risk cohort of, $n=254$, healthy patients and, $n=75$, diagnosed cases of NPC.

Anatomy /NPC Primary

The nasopharynx is bordered superiorly by the skull base, inferiorly by the oropharynx, anteriorly by the posterior choanae, posteriorly by the clivus and the first two cervical vertebrae, and latterly by fascia and the Eustachian tubes. Respiratory epithelium covers the nasopharynx and most NPC originate in Rosenmüller's fossa, located at each posterolateral aspect of the nasopharynx.

Figure: 1, Posterior nasopharynx showing specimen collection site for **NP Screen®**.



Clinical Specificity–Utility of the Ubiquitous EBV as a Marker for NPC

EBV is the causative agent for infectious mononucleosis and is associated with several head and neck malignancies such as inverted sinonasal papillomas, laryngeal carcinoma, certain parotid tumors and nasopharyngeal carcinoma as well as a wide spectrum of other human malignancies. The utility of EBV as a NPC marker relies on the fact that despite its ubiquitous nature EBV is almost never present, beyond incidental background levels, in or on the nasopharyngeal mucosa of a healthy patient [31-32,43-47] and, in high-risk populations nasopharyngeal malignant tumors are virtually always NPC [12], of Type III (95%), and Type II (5%) [4,7,8].

Clinical Studies

Clinical studies were conducted at referral clinics in Chinese communities in Toronto and Hong Kong. A cohort ($n=341$) of Chinese patients over 20 years of age not previously treated for NPC. Eligible patients were selected on the day of their visit to the clinic and their diagnostic status was not known prior to recruitment. The final cohort ($n=329$) consisted of 123 females with an average age of 53 years ranging from 29 to 82 years, and 206 males with an average age of 52 years ranging from 32 to 75 years. Eleven samples could not be processed due to low gDNA yield and, the brushing procedure for one patient was not completed due to gag response. The brushing procedure produced no significant complaints and minimal or no bleeding was noted after the procedure. All patients were discharged home uneventfully after the brushing procedure with no subsequent complications. As standard of practice, after the brushing procedure the post-nasal space was visualized and rated by an experienced ENT surgeon/Otolaryngologist and patients with suspicious clinical findings were referred for biopsy and histopathologic evaluation. The clinical performance for **NP Screen®** assay is provided below. In this analysis equivocal results are treated as false positive results.

Table 1, **NP Screen®** Screening Performance

	TP	FN	TN	FP	Sensitivity	Specificity	PPV NPV
Initial Status	71	1	247	10	98.6% 95%CI 92.5 to 100%	96.1% 95%CI 93.0 to 98.1%	87.7% 99.6%
Final Status	74	1	253	1	98.7% 95%CI 92.8 to 100%	99.6% 95%CI 97.8 to 100%	98.7% 99.6%

TP=True Positive, FN=False Negative, TN=True Negative, FP=False Positive, PPV=Positive Predictive Value, NPV=Negative Predictive Value

Resolved (9) and Unresolved Cases (1)

Initially 10 patients were positive by **NP Screen**® with no other clinical evidence for NPC (FP). Three (3/10) of these patients eventually presented clinically for NPC, six (6/10) of these patients resolved to normal on retest (TN), the remaining one (1/10) patient maintains his elevated EBV status on retest with no other clinical evidence for NPC (FP). The improvement in PPV from 87.7% to 98.7% (ref: Table 1) validates the clinical importance for equivocal case follow up.

Expected Values and Risk Assessment

The **NP Screen**® assay EBV detection level (EDL)² cut-off values and their interpretation are presented in Table 2. EDL Cut-off values were established using clinical trial data.

Table 2, Interpretation of **NP Screen**® assay results

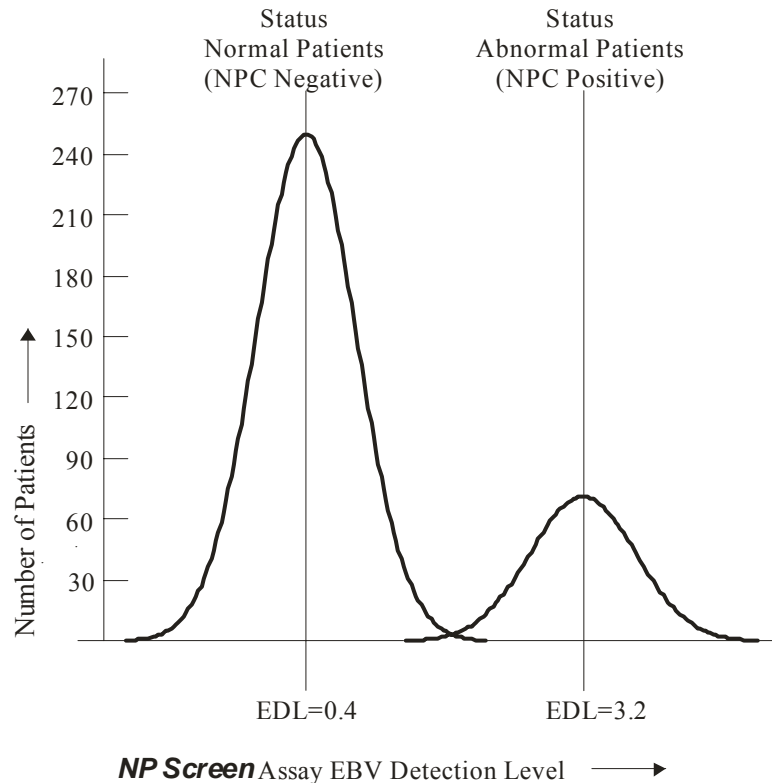
Assay Result	Status	Interpretation
EDL<1.7	Normal	Due to the ubiquitous nature of EBV, background incidental cell-free EBV may have been detected. EBV detection levels are consistent with the normal high-risk population. Low likelihood of EBV associated NPC. Results indicating normal do not preclude future abnormalities. In the absence of other clinical findings the patient is considered normal and should be tested at least annually. Based on clinical trials NP Screen ® has a Negative Predictive Value=99.6%.
1.7≤EDL≤2.6	Not Normal (Equivocal)	Results in this interval exceed normal background EBV detection levels and may indicate pervasive cell-free EBV or an underlying carcinoma. Persistent, reactivated EBV infections are believed to be antecedent to the development of NPC. A patient with an assay result within this interval must be recalled and retested after 6 to 8 weeks . The brushing instructions provided in Px500.50.03 Product Insert should be reviewed. Patients with assay results, which persist within this interval, should be referred for further clinical investigations or monitored with follow up testing at least semi annually. During clinical trials 3% of patient results (10 patients) were initially equivocal and on retest 6 patients resolved to normal, 1 patient maintained equivocal NP Screen ® results with no clinical evidence for NPC and, 3 patients developed NPC.
EDL>2.6	Not Normal (Abnormal)	Significantly elevated EBV detection level than that found in the normal high-risk population and is consistent with nasopharyngeal carcinoma. Assay results may be used in conjunction with other clinical presentations to assess a patient's need for a confirmatory procedure. Based on clinical trials NP Screen ® has a Positive Predictive Value=98.7%.

Patient samples are tested in duplicate and the subordinate EDL result is reported. The clinical significance of EDL values below the abnormal cut-off or above the normal cut-off, which respectively represent increasing magnitudes and decreasing magnitudes of EBV DNA levels in the starting template, has not been established.

² EDL is without units and represents the relative EBV DNA concentration level in the test sample.

Characterization of NPC Positive and Negative Patients Using **NP Screen**® Assay Results
EBV load profiles for nasopharyngeal samples taken from normal and abnormal sub groups in the high-risk for NPC population are characterized in Figure 2. The minor overlap of the distribution tails produces the equivocal zone.

Figure 2, Typical Distribution and Distribution Means for **NP Screen**® results for NPC Negative and Positive Patients in ENT Referral Clinic.



Conclusion

EBV is an appropriate marker for NPC and detection of EBV levels in specimens derived from the posterior nasopharynx of high-risk patients using the **NP Screen**® methodology is a useful aid to predict risk for NPC.

Warnings, Precautions and Limitations

The performance characteristics of NP Screen were established through validation by Primex Clinical Laboratories, and no approval is required by the U.S. Food and Drug Administration (FDA). Primex Clinical Laboratories is regulated under the Clinical Laboratory Improvement Amendments of 1988 ("CLIA") as qualified to perform high complexity clinical testing.

NP Screen® assay is for *in vitro* use only.

The collection kit is stable to the end of the month indicated by the expiration date. Confirm the expiry date, do not use expired kits.

Clinicians must follow the trans-oral brushing procedure provided in the product insert.

NP Screen® assay is only valid when specimen is collected using the materials provided collection kit. Performance of the assay with other specimen types or other collection methods has not been evaluated.

The results obtained with **NP Screen®** serves only as an aid to diagnosis and **should not** be interpreted as diagnostic themselves. Formal confirmatory nasopharyngeal biopsy and histopathologic analysis is required for diagnosis of cancer. Diagnosis shall not be solely based on **NP Screen®** assay results.

NP Screen® has not been evaluated for use with patients previously treated for NPC or patients with NPC recurrence or patients who have other risk factors, which may materially affect viral loading such as immuno-compromised patients.

NP Screen® assay has not been evaluated for, and is not intended for, monitoring the progression of NPC therapy efficacy.

NP Screen® assay has only been evaluated on patients of Chinese ethnicity. The use of **NP Screen®** assay with other population groups has not been evaluated.

The results of **NP Screen®** assay are not intended to prevent patients from proceeding for nasoendoscopy or biopsy. Results of the test should be interpreted in conjunction with other clinical findings.

Due to technical errors during sample collection or laboratory processing, a negative result does not exclude the possibility of EBV associated NPC.

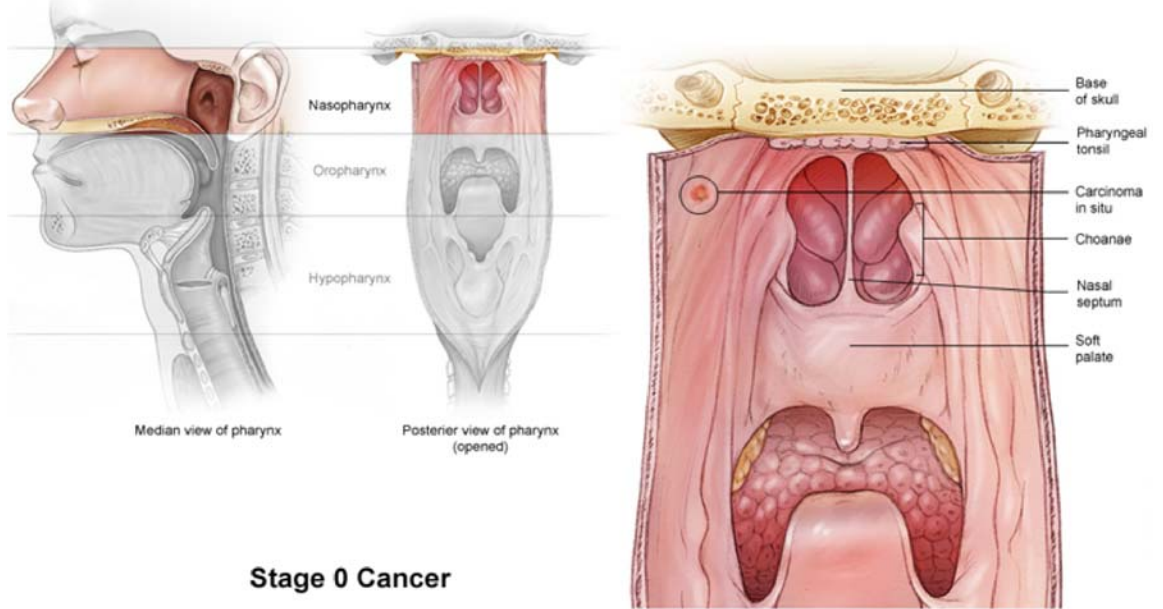
Patients with the flu, colds, sore throats or other upper respiratory tract conditions with productive phlegm or mucus **should not** be tested until the condition has resolved. Phlegm and mucus may transport EBV from other anatomical regions to the posterior nasopharynx and produce false positive or equivocal results.

NP Screen® assay may detect very early, microscopic or occult (sub mucosal) presentations for NPC, which may not be confirmed by biopsy and therefore the **NP Screen®** result may be classified as a false-positive. Patients with false positive or equivocal results should be followed up more frequently, with additional trans-oral brushings in conjunction with other clinical investigations.

Appendix A (Carcinoma in-situ)³

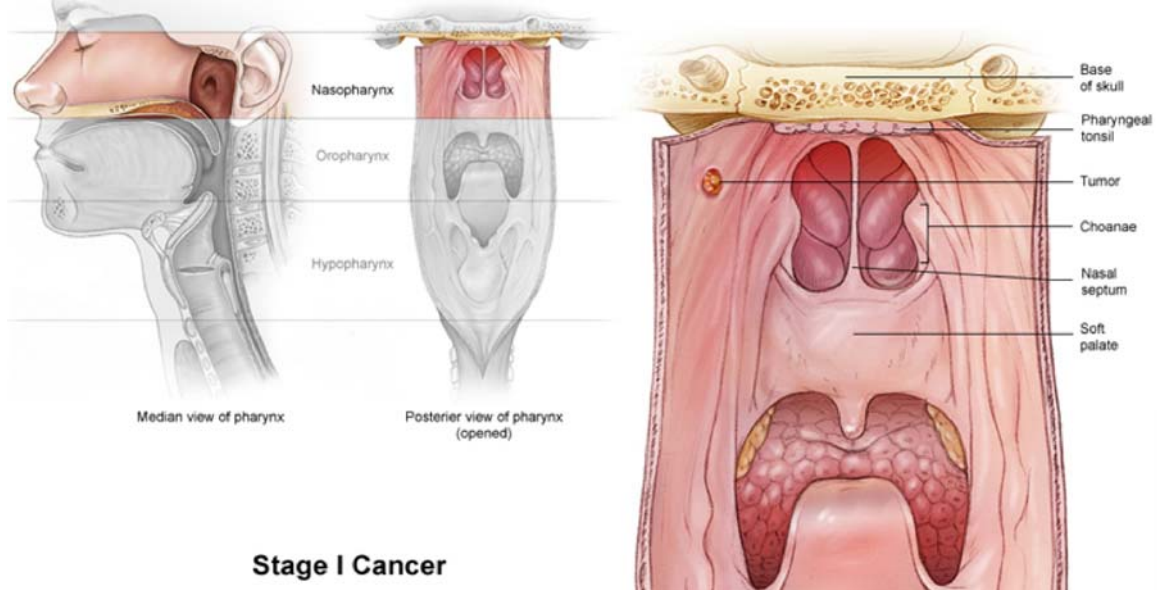
Stage 0

Indicates NPC in-situ (Tis), with no spread to lymph nodes (N0) or distant metastasis (M0).



Stage 1

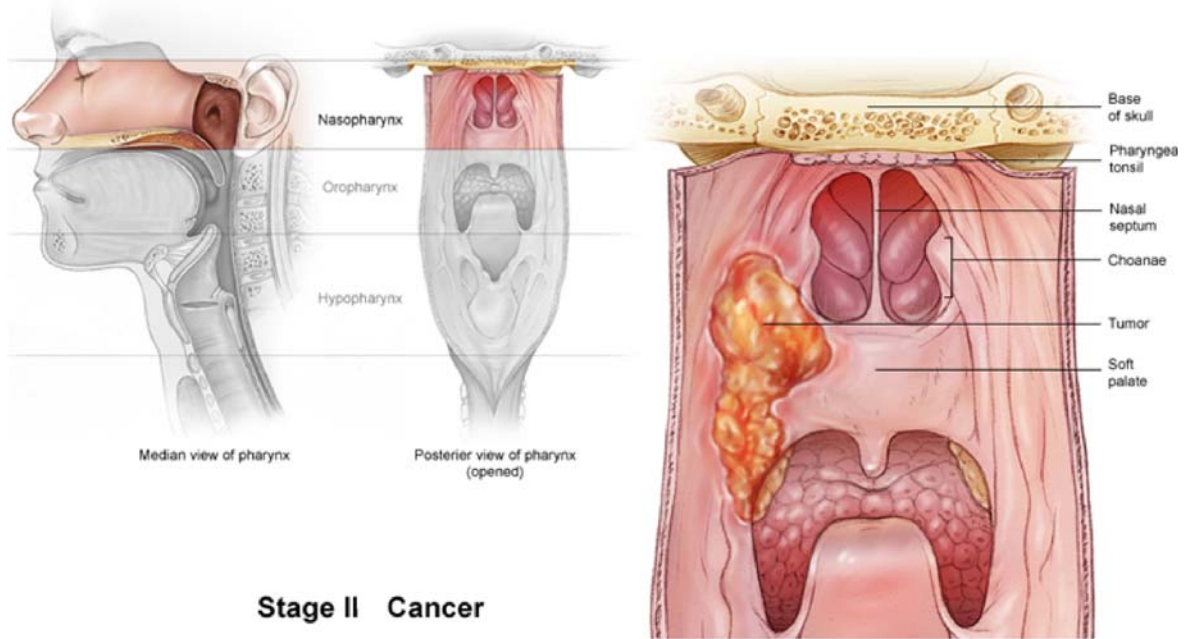
Indicates a small tumor (T1), with no spread to lymph nodes (N0) and no distant metastasis (M0).



³ American Society of Clinical Oncology

Stage 2

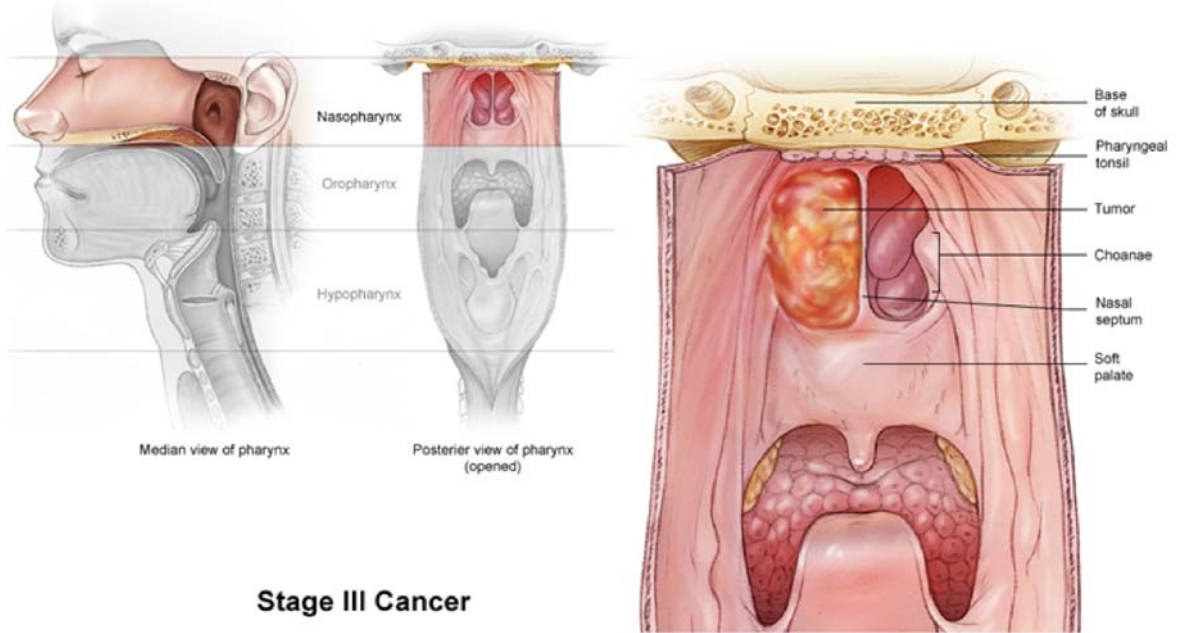
Describes a tumor that has extended beyond the nasopharynx (T2), but has not spread to lymph nodes (N0) or to distant parts of the body (M0).



Stage II Cancer

Stage 3

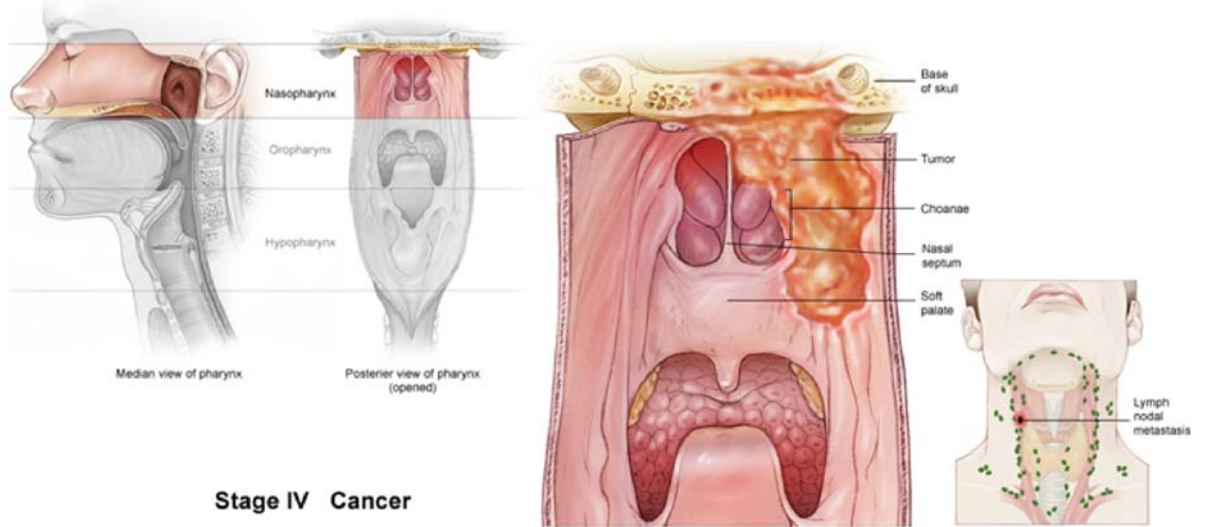
Describes noninvasive and invasive tumors (T1 or T2) that have spread to lymph nodes (N1, N2), but have not metastasized (M0), or larger tumors (T3) with or without nodal involvement (N0, N1, or N2) and no metastasis (M0).



Stage III Cancer

Stage 4 (Metastasis)

Describes any invasive tumor (T4) with either no lymph node involvement (N0) or spread to only a single same-sided lymph node (N1), but no metastasis (M0). It is also used for any cancer (T) with more significant nodal involvement (N2), but no metastasis (M0).



References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA: Cancer J Clin* 2005;55:74 – 108.
2. Ho JHC. Genetic and environmental factors in nasopharyngeal carcinoma. In: Nakahara W, Nishioka K, Hirayama T, Ito Y, editors. *Recent advances in human tumor virology and immunology*. Tokyo: University of Tokyo Press; 1971. p. 275 – 95.
3. Sugano H, Sakamoto G, Sawaki S, Hirayama T. Histopathological types of nasopharyngeal carcinoma in a low-risk area: Japan. In: de The' G, Ito Y, editors. *Nasopharyngeal carcinoma: etiology and control*. IARC scientific publications no. 20. Lyon: IARC; 1978. p. 27 – 39.
4. Zong YS, Zhang RF, He SY, Qiu H. Histopathologic types and incidence of malignant nasopharyngeal tumors in Zhongshan County. *Chin Med J (Engl)* 1983;96:511 – 6.
5. Levine PH, Connelly RR. *Epidemiology of nasopharyngeal carcinoma*. In: Wittes RE, editor. *Head and neck cancer*. New York: John Wiley & Sons; 1985. p. 13– 34.
6. Shanmugaratnam K, Sobin LH. *Histological typing of tumours of the upper respiratory tract and ear*. 2nd ed. Berlin: Springer-Verlag; 1991.
7. Yu MC, Henderson BE. Nasopharyngeal cancer. In: Schottenfeld D, Fraumeni JF, Jr., editors. *Cancer epidemiology and prevention*. 2nd ed. New York: Oxford University Press; 1996. p. 603– 18.
8. Vaughan TL, Shapiro JA, Burt RD, et al. Nasopharyngeal cancer in a lowrisk population: defining risk factors by histological type. *Cancer Epidemiol Biomarkers Prev* 1996;5:587 – 93.
9. Levine PH, Pocinki AG, Madagan P, Bale S. Familial nasopharyngeal carcinoma in patients who are not Chinese: *Cancer* 1992;70:1024-9
10. Abdel Hamid M, Chen JJ, Constantine N, Massoud M, Raab- Traub N. 1992 EBV strain variation: geographical distribution and relation to disease state. *Virology* 1992; 190: 168- 75.
11. Wang ZJ, Ramcharan S, Love EJ. Cancer mortality of Chinese in Canada. *Int J Epidemiol*: 1989; 18: 17-21.
12. Chang ET, Adami H, The Enigmatic Epidemiology of Nasopharyngeal Carcinoma; *Cancer Epidemiol Biomarkers Prev* 2006; 15(10), Oct 2006
13. Low WK, and Leong JL; Correlating clinical appearance of NPC with tumour staging: *J.R.Coll.Surg.Edinb.*, 45, June 2000, 146-147
14. Sham JS, Wei WL, , Kwan WH, Chan CW, Choi PH, Choy D: Fiberoptic Examination and biopsy in determining the exact extent of nasopharngeal carcinoma: *Cancer* 1989; 64:1838-42
15. Lee, A. W. M., Foo, W., Law, S. C. K., Poon, Y. F., O, S. K., Tung, S. Y., Sze, W. M., Chappell, R., Lau, W. H., and Ho, J. H. C. Staging of nasopharyngeal carcinoma: from Ho's to the new UICC system. *Int. J. Cancer*, 84: 179–187, 1999.
16. Chang YS, Tyan YS, Liu ST, Tsai MS, Pao CC. Detection of Epstein-Barr virus DNA sequences in nasopharyngeal carcinoma cells by enzymatic DNA amplification. *J Clin Microbiol* 1990;28:2398-2402.
17. Wu TC, Mann RB, Epstein JI, et al. Abundant expression of EBER1 small nuclear RNA in nasopharyngeal carcinoma: a morphologically distinctive target for detection of Epstein-Barr virus in formalin-fixed paraffin-embedded carcinoma specimens. *Am J Pathol* 1991;138:1461-1469.
18. Chen CL, Wen WN, Chen JY, Hsu MM, Hsu HC. Detection of Epstein-Barr virus genome in nasopharyngeal carcinoma by in situ DNA hybridization. *Intervirolgy* 1993;36:91-98.
19. Pathmanathan R, Prasad U, Chandrika G, Sadler R, Flynn K, Raab-Traub N. Undifferentiated, nonkeratinizing, and squamous cell carcinoma of the nasopharynx: variants of Epstein-Barr virus-infected neoplasia. *Am J Pathol* 1995;146:1355-1367.
20. Lee WY, Hsiao JR, Jin YT, Tsai ST. Epstein-Barr virus detection in neck metastases by in-situ hybridization in fine-needle aspiration cytologic studies: an aid for differentiating the primary site. *Head Neck* 2000;22:336-340.
21. Tsai ST, Jin YT, Su IJ. Expression of EBER1 in primary and metastatic nasopharyngeal carcinoma tissues using in situ hybridization: a correlation with WHO histologic subtypes. *Cancer* 1996;77:231-236.

22. Macdonald MR, Freeman JL, Hui MF, et al. Role of Epstein-Barr virus in fine-needle aspirates of metastatic neck nodes in the diagnosis of nasopharyngeal carcinoma. *Head Neck* 1995;17:487-493.
23. Chao TY, Chow KC, Chang JY, et al. Expression of Epstein-Barr virus-encoded RNAs as a marker for metastatic undifferentiated nasopharyngeal carcinoma. *Cancer* 1996;78:24-29.
24. Raab-Traub N, Flynn K, Pearson G, et al. The differentiated form of nasopharyngeal carcinoma contains Epstein-Barr virus DNA. *Int J Cancer* 1987;39:25-9.
25. Desgranges C, Wolf H, De-Thé G, et al. Nasopharyngeal carcinoma. X. Presence of Epstein-Barr genomes in separated epithelial cells of tumors in patients from Singapore, Tunisia and Kenya. *Int J Cancer* 1975;16:7-15.
26. Raab-Traub N, Flynn K. The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. *Cell* 1986;47:883-9.
27. Yates JL, Guan N, Epstein-Barr virus derived plasmids replicate only once per cell cycle and are not amplified after entry into cells. *J Virol* 1991;65:483-8.
28. Niedobitek G, Young LS. Epstein-Barr virus persistence and virus associated tumors. *Lancet* 1994;343:333-5
29. Pathmanathan R, Prasad U, Sadler R, Flynn K, Raab-Traub N. Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. *N Engl J Med* 1995; 333:693-8.
30. Lawrence YS et al, Epstein-Barr virus Infection: Basis of Malignancy and Potential for Therapy. *Expert Reviews in Molecular Medicine*, ISSN 1462-3994 Cambridge University Press 2001
31. Sam, CK et al, Analysis of Epstein-Barr virus infection in nasopharyngeal carcinoma. *International Journal of Cancer* 53, 957-962, PubMed ID: 93231699.
32. Liavaag PG, Cheung RK, Kerrebijn JD, Freeman JL, Irish JC, Dosch HM. The physiologic reservoir of Epstein-Barr virus does not map to upper aerodigestive tissues. *Laryngoscope* 1998; 108:42-6.
33. Gulley, ML, Review, Molecular Diagnosis of Epstein-Barr virus Related Diseases. *Journal of Molecular Diagnostics*, Vol 3, No 1, Feb 2001.
34. Glenna BW, Mononucleosis and Epstein-Barr virus Infection, *eMedicine* Sept, 29, 2005.
35. Chien YC, Chen JY, Lui MY, Yang HI, Hsu MM, Chen CJ, et al. Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. *N Engl J Med* 2001;345:1877-1882.
36. Zeng Y, Zhang LG, Wu YC, et al, Serological Markers of Epstein-Barr virus Infection and Nasopharyngeal Carcinoma in Wuzhou City, China, (1982) *International Journal of Cancer* 1982; 29:139-141
37. Deng H, Zhao Z, Zhang Z, (1995) Serological Screening on Nasopharyngeal Cancer in 338,868 Persons in 21 Cities and Countries of Guangxi Region, China, (1995) *Zhonghua Yu Fang Yi Xue Za Zhi*, 1995;29:342-343
38. Zeng Y, Zhong JM, Li LY, et al. Follow-Up Studies on Epstein-Barr virus IgA/VCA antibody positive persons in Zangwu County, China, (1983) *Intervirology* 1983;20:190-194
39. Zong YS, Sham JS, Ng MH, Ou XT, Guo YQ, Zheng SA, Liang JS, Qiu H. 1992 Immunoglobulin A against viral capsid antigen of Epstein-Barr virus and indirect mirror examination of the nasopharynx in the detection of asymptomatic nasopharyngeal carcinoma. *Cancer*; 69: 3- 7.
40. Chen JY, Chen CJ, Liu MY, Cho SM, Hsu MM, Lynn TC, et al. Antibody to Epstein-Barr virus specific Dnase as a Marker for field Survey of Patients with Nasopharyngeal Carcinoma in Taiwan, (1982) *Jouranl Med Viology* 1989;27:269-273
41. Wei, WI et al., Outcome of Patients With positive Epstein-Barr virus Serologic Status in the Absence of Nasopharyngeal Carcinoma in Hong Kong. *Ach Otolaryngol Head and Neck Surg*, Vol 130, June 2004.
42. Tune C.E., Liavang P.-G, Freeman J.L., van den Brekel M.W., Shpitzer T, Kerrebijn J.D.F., Payne D, Irish J.C., Ng R, Cheung R.K., Dosch H.-M., Nasopharyngeal Brush Biopies and Detection of Nasopharyngeal Cancer in a High-Risk Population, *Journal of The National Cancer Institute*, May 1999.

43. Feinmesser R, Miyazaki I, Cheung R, Freeman JL, Noyek AM, Dosch HM. Diagnosis of nasopharyngeal carcinoma by DNA amplification of tissue obtained by fine needle aspiration. *N Engl J Med* 1992;326:17-21.
44. MacDonald MR, Freeman JL, Hui MF, Cheung RK, Warde P, McIvor NP, et al. Role of Epstein-Barr virus in fine needle aspirates of metastatic neck nodes in the diagnosis of nasopharyngeal carcinoma. *Head and Neck* 1995;17:487-93.
45. Feinmesser R, Feinmesser M, Freeman JL, Noyek AM, Livni N. 1992 Detection of occult nasopharyngeal primary tumours by means of in situ hybridization. *J Laryngol Otol* ; 106: 345-8.
46. Sam CK, Brooks LA, Niedobitek G, Young LS, Prasad U, Rickinson AB. Analysis of Epstein-Barr virus infection in nasopharyngeal biopsies from a group at high risk of nasopharyngeal carcinoma. *Int J Cancer* 1993;53:957-62.
47. Faulkner GC, Burrows SR, Khanna R, Moss DJ, Bird AG, Crawford DH. X-linked agammaglobulinemia patients are not infected with EBV: implications for the biology of the virus. *J Virol* 1999;73:1555-64.
48. Leung S, Tam JS, Chan ATC, Zee B, Chan LYS, Huang DP, Hasselt AV, Johnson PJ, Lo YMD. Improved accuracy of detection of nasopharyngeal carcinoma by combined application of circulating EBV DNA and anti-EBV capsid antigen IgA antibody. *Clinical Chemistry* 50:339-345, 2004.
49. Zong YS, Sham JS, Ng MH, Ou XT, Guo YQ, Zheng SA, Liang JS, Qiu H. Immunoglobulin A against viral capsid antigen of Epstein-Barr virus and indirect mirror examination of the nasopharynx in the detection of asymptomatic nasopharyngeal carcinoma. *Cancer*; 1992;69: 3-7.
50. Wei WI, Sham JS, Zong YS, Choy D, Ng MH. The efficiency of fiberoptic endoscope examination and biopsy in detection of early nasopharyngeal carcinoma. *Cancer* 1991;67:3127-30
51. Baer R, Bankier A, Biggin M, Deininger P, Farrell P, Gibson T, Hatfull G, Hudson G, Satchwell S, Sequin C, Tuffnell P, Barrell B. DNA sequence and expression of the B95-8 Epstein-Barr virus. *Nature* 310:201-211, 1984.