temperature cycles were as follows: 40 cycles of 30 s at 95°C, 1 min at 57°C, and 1 min at 72°C.

The detection limit was estimated on serial dilutions, from 10^8 to 10^1 copies/μL, of cloned HPV MY09/11 and the C. trachomatis CTP PCR product in the pCR® 2.1-TOPO® (Invitrogen Ltd). To evaluate the sensitivity and specificity of the multiplex PCR for the detection of each organism, we performed single PCRs on all samples: one PCR that detects HPV by use of the GP5+/6+ primers (10), generating a 150-bp product; and one that detects C. trachomatis by use of primers specific to the hsp60 gene of C. trachomatis (sense, 5'-GAT GGT GTT ACC GGT GCG A-3'; antisense, 5'-TAA TCG TCT TTA ACA ACC T-3'), generating a truncated version (309 bp) of a previously described product (13). PCRs were also performed with the MY09/11 and CTP1/2 primer sets singly.

In the screened population, 21% (21 of 100) of samples were positive for HPV by the multiplex PCR (Table 1). The MY09/11 primers identified 3 other samples as HPV positive that were not detected by the multiplex assay (Table 1). Either the MY09/11 or the GP5+/6+ primers confirmed all 21 of the samples positive by the multiplex assay. Two samples were positive for C. trachomatis in the multiplex assay (Table 1). These were confirmed positive by the CTP1/2 and Hsp60 primers in single PCRs, and no additional positive samples were detected. A positive sample was defined as positive by either the multiplex or any of the single PCRs for that organism. The sensitivity and specificity of the multiplex with respect to single PCR for the detection of HPV in the opuntioanlly screened samples were 89% (95% confidence interval, 70%–97%) and 100% (94%–100%; Table 1). The multiplex assay was 100% specific and sensitive for the detection of C. trachomatis with respect to single PCR in the screened population (Table 1).

To estimate the diagnostic sensitivity of the multiplex assay for the detection of C. trachomatis, we performed multiplex PCR on 34 Preserv-
enlargements. A combined positron emission tomography/computed tomography whole body scan confirmed left parotid involvement as well as supra- and infradiaphragmatic involvement of the lymph nodes. Laboratory investigation showed hemoglobin, leukocyte count and differentiation, and a platelet count within the appropriate reference intervals. Serum lactate dehydrogenase was increased. The IgG concentration was 5.46 g/L, the IgM concentration 1.27 g/L, and the IgA concentration 0.78 g/L. CZE results were normal, but immunofixation revealed a monoclonal heavy chain. A lymph node biopsy showed an extranodal marginal zone B-cell lymphoma of mucosa-associated tissue. The patient was treated with chlorambucil and had an excellent response.

The CZE electropherogram [Pargon 2000 (software version 1.6.02); Beckman-Coulter; Fig. 1] revealed a monoclonal heavy chain disease. Lane ELP, electrophoresis with no immunofixation. CZE was performed on a Pargon 2000 (software version 1.6.02), and immunofixation was on a Sebia system with Hydragel 4 IF gels. 

CZE may fail to detect small monoclonal proteins (4) and (b) that monoclonal free heavy chains are frequently not detected (in >50% of cases) by routine serum protein electrophoresis (3). CZE may miss this specific monoclonal heavy chain because of its low concentration and/or its migration in the α2-globulin region. It is therefore essential to perform immunofixation on all samples (serum and urine) in which there is high clinical suspicion of the presence of a monoclonal protein.