Dear Editors,

We value all comments on our research, and the opportunity to provide further information on our study describing the HPV16-specific tumour marker DRH1 [1].

We used an assay that was CE certified under the European In-Vitro Diagnostic Devices Directive, implying compliance with regulatory requirements on documentation and technical validation. This includes assessments of test reproducibility. Significant bias from batch effects can therefore be excluded.

Specificity of the assay was assessed in a large control population (n = 1064*, see Table 1) of randomly selected, CRP-negative (<1 mg/l), HIV- and hepatitis-negative blood donors without history of cancer and of unknown HPV-vaccination status. Controls and cases were recruited within the same time frame and geographical region (German-speaking countries). A broad range of ages was tested, with the largest proportion comprised of 30–70-year-olds to reflect the age group most relevant for early cancer detection (Fig. 1a). Separate specificity analyses were presented for subjects below and above 30 years, reflecting official recommendations on age for HPV testing in the context of cervical cancer. Contrary to the statement in the letter [3], ROC analysis was performed on the full sample. No subjects were excluded post-hoc and all analyses were subject to approval by an independent statistician as part of the review process. Following the Letter authors’ comments, we have repeated ROC analysis after

![Fig. 1.](image)

**Fig. 1.** a: Age- and sex-distributions of control population (n = 1064) and cancer group (n = 20). Red indicates a positive test result (HPV16-L1 DRH1 antibodies ≥1000 ng/ml). HPV vaccination status of the control population was not known, although nineteen of 25 positive results (76%) were observed in females <30 years, corresponding to the group most likely to be vaccinated.

b: Receiver-operating-characteristic analysis (ROC) for the serological detection of oropharyngeal cancer using a sex- and age-matched control group (n = 260) reveals improved performance (AUC 0.97, 95% CI 0.91, 1.0) compared to original analysis.

Table 1
Overview of serological test results by clinical diagnosis.

| Clinical diagnosis (HPV16-induced OPC) | positive | negative* |
|--------------------------------------|----------|-----------|
| Serological test (DRH1 at cut-off 1000 ng/ml) | 19       | 25        |
|                                      | 1        | 1039      |

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Table 2
Estimated impact of HPV-induced (pre)-cancer rates and DRH1 marker sensitivity and specificity on screening characteristics.

| Targeted cases                                      | Incidence per 100,000 | HPV16-attributable incidence per 100,000 | Marker sensitivity | Marker specificity | Detected cases per 100,000 screened | False positives per 100,000 screened | Estimated PPV | Number to screen to detect 1 case |
|-----------------------------------------------------|-----------------------|------------------------------------------|-------------------|-------------------|-------------------------------------|--------------------------------------|--------------|---------------------------------|
| Oropharynx and anogenital cancers                   | 20                    | 10                                       | 90%               | 99.5%             | 9                                   | 491                                  | 2%           | 11,111                          |
| Oropharynx, anogenital cancers, and CIN2+            | 323                   | 161                                      | 90%               | 99.5%             | 145                                 | 355†                                  | 29%          | 690                             |

* detected cases, false positives, estimated PPV and number to screen have been calculated as described in Waterboer et al., letter to the editor, EBioMedicine [3].
+ based on published data by the HPV Information Centre and the German guidelines on cervical cancer prevention [4].
+ anogenital cancer sites include: cervix, anus, penis, vulva, vagina.
+ CIN2+, cervical intraepithelial neoplasia of grades 2 or higher [4]. Cases of pre-cancerous lesions at other anogenital sites have not been included due to lack of representative incidence data.
+ HPV16-induced pre-cancerous lesions of the anus, penis, vagina, vulva and OPC have not been accounted for in the incidence rate due to lack of representative incidence data.

We believe that a collaborative approach would be beneficial for the field moving forward. There would be particular benefit in evaluating the DRH1 assay alongside the E6-based assay in a well-characterised study population.

When, how, and for which patient groups these findings are moved into clinical practice is a question that needs to be considered in dialogue with a wide range of disciplines, including physicians, payers, and policymakers.

Author contributions
All authors contributed equally to this work.

Declaration of Competing Interest
All authors declare no conflict of interest related to this work.

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