Provided that mRNA is expressed exclusively in tumor tissue and not by normal nucleated blood cells, its presence outside the organ identifies already disseminated cells. We have recently found hematogenous spread of prostate epithelial cells during radical prostatectomy [2] as well as during the course of transurethral resection of the prostate (unpublished data). The different mechanisms underlying hematogenous dissemination of cells from prostate gland remain largely unknown. Among the postulated factors are proteases, hypervascularization, growth factors, and impaired expression of adhesion factors (for review, see [3]). Among the diverse interactions during tumor proliferation, we believe inflammatory processes are likely to be among the key events that drive prostate cells from the acini to the blood vessels.

Between December 1995 and June 1996, we investigated 24 patients with acute bacterial prostatitis (ABP), using a prostate-specific membrane antigen (PSMA) nested reverse transcriptase (RT)-PCR assay [4] to detect prostate epithelial cell dissemination in peripheral blood. The clinical diagnosis of ABP was based on findings of dysuria, pollakiuria, marked malaise, high fever (>40 °C), chills, and prostate pain during digital rectal examination (DRE). The patients’ mean age was 37 years (range 24–45 years), and their mean serum PSA (Hybritech® assay) was 23.4 μg/L (range 8.9–63.2 μg/L). Escherichia coli was found in the urine of 17 patients; Enterobacter cloacae, Citrobacter freundii, and Proteus mirabilis were each found in 1 patient. Cytobacterial urine examinations remained negative for 4 patients. All patients were successfully treated with 400 mg of ofloxacin daily for 3 weeks.

Evidence of hematogenous dissemination of prostate epithelial cells was found in 41% of the ABP patients. PSMA RT-PCR positivity appears to be significantly more common in ABP patients (Fisher’s exact test, \( P < 0.05 \)) in that none of the 12 healthy volunteers tested for a non-specific effect of DRE were positive for this assay (Table 1). Because PSMA transcripts are not ordinarily found in polymorphonuclear cells under normal circumstances [4], the presence of an acute infection may increase the baseline of PSMA mRNA expression. To test this hypothesis, we used PSMA RT-PCR to test blood samples drawn from 10 female patients with bacterial sepsis (leukocytes >20 G/L) and found negative results in all cases.

ABP is associated with inflammation in part or all of the prostate gland [5]. Acute inflammation may promote hematogenous spread of prostate cells. Epithelial cells can migrate out of the prostate after (a) matrix destruction by polymorphonuclear leukocytes within neighboring acini and (b) hypervascularization because of the local inflammatory response. The occurrence of prostate epithelial cells in blood of noncancer patients such as ABP patients further emphasizes that nested RT-PCR protocols based on tissue-specific effect of DRE were positive for this assay (Table 1). Because PSMA transcripts are not ordinarily found in polymorphonuclear cells under normal circumstances [4], the presence of an acute infection may increase the baseline of PSMA mRNA expression. To test this hypothesis, we used PSMA RT-PCR to test blood samples drawn from 10 female patients with bacterial sepsis (leukocytes >20 G/L) and found negative results in all cases.

Table 1. Evidence of hematogenous dissemination of prostate epithelial cells.

<table>
<thead>
<tr>
<th>n</th>
<th>Healthy volunteers</th>
<th>ABP patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>12 (23–36)</td>
<td>24 (24–45)</td>
</tr>
<tr>
<td>Serum PSA, μg/L</td>
<td>&lt;4</td>
<td>23.4 (8.9–63.2)</td>
</tr>
<tr>
<td>PSMA RT-PCR positive results, no. (%)</td>
<td>0 (0)</td>
<td>10 (41.6)</td>
</tr>
</tbody>
</table>

Mean (range) of results is shown.

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References


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Misleading Results with Opus®

To the Editor

We recently found unsatisfactory agreement of luteinizing hormone (hLH) results of the hLH Spec assay (Wallac) and Opus Magnum hLH assay (Behring Diagnostics) (Fig. 1A). Particularly disturbing were the occasional marked discrepancies.

According to the manufacturers, both assays are standardized against the WHO 2nd International Standard for pituitary hLH for immunoassay (code 80/552). Both assays are claimed to be highly specific, with cross-reactivities for hCG of <0.02%


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To the Editor:
Glycoprotein hormones like LH occur as a complex mixture of circulating isoforms. Standardization and agreement among immunoassays is notoriously problematic, most probably attributable to the use of highly specific but different monoclonal antibodies in the various assays. Recent publications cited by Eskola-Williams above have demonstrated that certain LH tests underestimate or even completely fail to detect LH in some individuals who were shown by functional bioassays to have physiologically active molecules. Nucleotide mutations within codons 8 and 15 in the LH β-subunit gene have been described and appear to account for the majority of these immunologically unrecognizable LH isoforms. The frequency of this variant form of LH has been shown to be relatively high in the Finnish population, ~28%.

The Opus™ LH immunoassay (Behring Diagnostics) utilizes a polyclonal/monoclonal antibody pair, both specific to the β-subunit. The monoclonal antibody was selected for its high degree of specificity for LH. Method comparison studies

(AutoDELFIA) and <0.12% (Opus Magnum); their respective detection limits are 0.05 and 0.6 IU/L. Among 35 samples (Fig. 1A) striking discrepancies were seen in 3, for which the Opus results were below the stated detection limit (0.6 IU/L) whereas the DELFIA results were 5.3, 26.1, and 35.3 IU/L.

An immunologically anomalous, biologically active common genetic variant has been described in the Finnish population [1–3] and recently in other populations as well [4–6]. The frequency of the variant, either heterozygous or homozygous, is 8–30% [7].

For further analyses we sent the samples to Kim Pettersson at the University of Turku. Use of the double-monoclonal I3/A2 intact LH assay described by that laboratory [2] yielded an excellent correlation with the results from the Opus Magnum assay (Fig. 1B). This double-monoclonal I3/A2 intact LH assay, in which the capture antibody detects an epitope present only in the intact dimer whereas the tracer antibody is directed against the alpha subunit, has been shown to be totally unreactive with the hLH variant [8]. As shown in Fig. 1C and D, hLH in the three anomalous samples did not react in this assay, indicating a homozygous variant hLH; 9 other samples from the 35 women appeared to be heterozygous. The data in Figs. 1C and 1D suggest that the Opus Magnum assay uses at least one antibody similar to those in the double-monoclonal I3/A2 intact LH assay.

References

Fig. 1. Comparison of results for samples from 35 fertile women: (A) Opus Magnum LH assay vs AutoDELFIA LH Spec assay (y = −0.985 + 0.778x, Sb = 0.093, Sy/x = 3.9); (B) Opus Magnum LH assay vs I3/A2 intact LH assay (y = −0.257 + 0.772x, Sb = 0.016, Sy/x =1.4); (C) ratio between I3/A2 intact LH assay and AutoDELFIA LH Spec assay results in relation to serum LH concentration determined by AutoDELFIA LH Spec; (D) ratio between Opus Magnum LH assay and AutoDELFIA LH Spec assay results in relation to serum LH concentration determined by AutoDELFIA LH Spec assay.

In C and D, the population is distributed into three categories: (□) homozygotes, (■) heterozygotes, and (×) normal type for the variant LH allele. Sb = standard error of slope, Sy/x = standard error of estimates.
were performed with several commercially available assays, including DELFIA, and initial correlation coefficients were quite good (all r > 0.9, many > 0.95). Additionally, serial blood samples were obtained from 20 different women throughout the course of their menstrual cycles and clinically evaluated for LH, follicle-stimulating hormone (FSH), and estradiol on the Opus and other commercial systems. The Opus LH assay correctly identified the follicular, ovulatory, and luteal phase of the menstrual cycle in all cases, peaking on the same day as or one day earlier than the estradiol peak. In certain instances, however, the recovery of LH was lower that in some other commercial kits. The low bias was not attributed to calibration because recoveries were variable within the same method comparison. Discrepancies in recovery were believed to result from the differential recognition of various LH isoforms in individual patients. We did not, however, observe any samples in which the Opus assay totally failed to recognize LH.

Variable recoveries among LH assays utilizing monoclonal antibodies are common and, given that the clinical utility of the assay was demonstrated, the absolute value for LH seemed less important. As literature accounts became increasingly available describing the LH variant commonly seen in the Finnish population, however, more investigation into the specificity of the Opus reagents was undertaken. Results suggest that the antibody used in our current kit does not recognize the LH variant described in the Finnish study conducted by Eskola-Williams. Why we did not observe the degree of discordance described in the Finnish study is probably related to the relatively low frequency of the variant in the population we sampled, and similar specificity of the reagents used in several of the other assays. Indeed, good correlation was observed when the Opus assay was compared with the I3/A2 “intact” LH assay described in the Finnish study.

We agree that the value of a substantial number of LH samples will be underestimated in the Finnish population. The number of samples testing negative (homozygotes) for LH, however, will be relatively small. The references cited by Eskola-Williams describe the 28% population frequency as consisting of 24% heterozygous and 4% homozygous. According to our studies with menstrual cycle panels, accurate clinical information regarding cycle phase can still be obtained in most cases. We also agree that failure to recognize physiologically active LH, even in a small subset of patients, is undesirable, and test results not agreeing with clinical and other biochemical data should be interpreted with caution. A second-generation Opus LH assay has been developed that recognizes the LH variant described here while retaining excellent specificity. This new assay is currently available for research evaluation and will be commercially available beginning in the second quarter of 1997.

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Parameters Relating to Ultrasensitive Prostate-Specific Antigen Assays

To the Editor:

Several recent papers have dealt with the performance of prostate-specific antigen (PSA) assays at very low concentrations [1–3]. In these papers, no less than 5 different parameters have been discussed: the lower limit of detection (LLD), the biological detection limit (BDL), functional sensitivity (FS), the residual cancer detection limit (RCDL), and the minimal detectable concentration (MDC). One may wonder whether such a profusion of terms is meaningful or necessary. However, I believe that, although these parameters are to some extent presented or used as alternatives to each other, fundamental differences exist among them. Here, I aim at distinguishing two groups of parameters, each of which has a specific use.

Two types of questions may arise. First, given a certain measured concentration, does this measurement signify the presence of PSA, as opposed to assay noise? In other words, does the reading of the assay allow to state that the concentration is different from zero? The approach to this question involves statistical inference. First, a distribution of measurements should be obtained (with the same method and under the same conditions) in multiple specimens known to have zero concentration of the analyte. When the analytical signal falls far to the right on the distribution (or to the left for calibration curves with negative slopes), it is unlikely that a sample with actual zero concentration would yield a signal equal to or higher than the one measured. Stated differently, it is thus unlikely that the sample contains no analyte. So, ascertaining the presence of PSA in a clinical sample always and exclusively involves a measurement limit based on the distribution of results from a specimen devoid of analyte. Two parameters qualify as such: the LLD and the RCDL. These differ only in the nature of the blank specimens used: zero calibrators in the case of LLD, and actual clinical samples for the RCDL. In the latter, evidence for the absence of PSA is based on clinical information.

In contrast, parameters such as FS, BDL, and MDC do not qualify as limiting values of a zero-sample distribution and therefore cannot be used to judge individual specimens. It is easy to see that FS does not take into account the distribution of null-specimens but only the variability in samples with very low concentrations. The BDL and the MDC do take into account the distribution of values in specimens devoid of analyte but also reflect variability in samples with very low concentrations.

A second type of question is, how does a given assay perform at very low concentrations? In this respect, limiting values such as LLD and RCDL reveal only part of the picture, because they relate only to blank