TO THE EDITOR:

Human semen is the secretion of the male reproductive organs, containing sperm cells and seminal plasma (SP), a complex mixture of testicular, prostatic, and accessory gland secretion that provides biochemical support for ejaculate function. Assessing the quality of human semen and its fertilizing capacity therefore requires not only qualitative and quantitative analysis of spermatozoa, but also biochemical analyses of the SP (1).

SP fructose is secreted by seminal vesicles during ejaculation, provides energy for sperm motility, and is a useful test in differential diagnosis of azoospermia. A rapid, simple, specific, and quantitative SP fructose photometric determination by hexokinase and phosphoglucose-isomerase using a semiautomated bichromatic analyzer was developed in our laboratory (2).

The aim of this study was to validate the performance of the enzymatic method for SP fructose on a Beckman Coulter (BC) AU400 analyzer (Olympus Mishima), as well as to challenge the performance of the method regarding quality claims required for accreditation by the International Organization for Standardization (ISO) 15189:2012 Medical Laboratories—Requirements for quality and competence.

SP fructose determination by the enzymatic method is carried out indirectly via glucose. After fructose phosphorylation with hexokinase, the resulting fructose 6-phosphate is converted into glucose 6-phosphate by phosphoglucone isomerase. Glucose 6-phosphate is further oxidized by glucose 6-phosphate dehydrogenase, yielding 6-phosphogluconate and NADH. The amount of NADH formed is stoichiometric with the amount of D-fructose and D-glucose, so the measured rate of NADH formation reflects quantitatively the amount of glucose and is equivalent to the amount of SP fructose. We used the following reagents: BC glucose hexokinase (BC Ireland), D-fructose analytical grade (Kemika), and Sigma-Aldrich phosphoglucose isomerase.

Leftover semen samples from males undergoing routine fertility evaluation (aged 28–41 years), with normal semen parameters (>60 × 10⁶ sperm/mL, motility >50%, and white blood cell count <10⁶/mL), were used in this study. Written informed consent was obtained from the participating subjects.

The semen samples were centrifuged immediately after liquefaction (900g, 10 min), and SP was removed for fructose analysis. The concentration of SP fructose was measured in manually diluted, fresh samples (redistilled water, 1:20) by an elective laboratory reference
according to universal concept certainty (MU) was calculated were within quality speci-

6-phosphate to glucose 6-phosphate during conversion of fructose ering the reaction that occurs method performance. Consid-

rers without fertility problems, additionally fructose was mea-

ured in 20 SP samples obtained from healthy volun-

tees without fertility problems, to confirm the previously estab-

lished reference intervals (8.3–27.8 mmol/L). All measured samples were within the refer-

ence interval; the results were 8.8–23.6 mmol/L.

In conclusion, the results of this verification study show that the automatic enzymatic determination of SP fructose on a BC AU400 analyzer met the desirable quality specifications and requirements for the accreditation according to the ISO 15189:2012 Standard, thereby providing a reliable diagnostic method for the clinical assessment of male infertility.

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vestigated and resolved.

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Manifestations of Instrument-to-Instrument Variability on an Internal Control During Real-Time PCR

TO THE EDITOR:

Using a laboratory-developed test, real-time PCR with the 3M Integrated Cycler (Focus Diagnostics) was used to amplify and detect in a single reaction the IS481 insertion sequence of *Bordetella pertussis*, the IS1001 insertion sequence of *Bordetella parapertussis*, and an internal control (IC) using channels that read FAM (*B. pertussis*), CAL Fluor Red 610 (*B. parapertussis*), and Quasar 670 (IC). The IC consisted of exogenous DNA, IC primers, and an IC probe.

A positive control containing a mixture of cultured *B. pertussis* (ATCC 9340) and *B. parapertussis* (ATCC 15311) was prepared and frozen in 215-μL aliquots. Patient specimens were nasopharyngeal swabs processed into M4 transport media or PBS. DNA extraction was performed on the Maxwell 16 instrument (Promega) using 200 μL control or patient sample, to which 5 μL IC DNA was added. PCR was performed on a 3M Integrated Cycler using a Universal Disc (Focus Diagnostics MOL1400). Each PCR contained 4 μL 2.5X Universal Master Mix (Focus Diagnostics MOL9010), 1 μL probe/primer [prepared from a mixture of 1 μL *B. pertussis* primer pair, 1 μL *B. parapertussis* primer pair, 0.5 μL IC DNA primer pair (Focus Diagnostics MOL9006, MOL9007, MOL9000)], and 5 μL DNA. Cycling conditions were 2 min, 97 °C, followed by 39 cycles of 10 s, 97 °C, and 30 s, 60 °C. Using one 3M Integrated Cycler over 11 months, crossing threshold (CT) reference ranges for the *B. pertussis*/parapertussis positive control were determined from 287 independent runs. The IC CT reference range was determined from 287 positive and 281 negative extracted controls. CTs are automatically determined by the 3M Integrated Cycler software at user-defined thresholds. Using average CTs ± 2–3 SDs, CT reference ranges of 25.3–29.0 (*B. pertussis*), 27.8–31.8 (*B. parapertussis*), and 27.4–34.0 (IC) were established.

While *B. pertussis*– and *B. parapertussis*–positive control CTs were highly reproducible, unexpected increases in the IC CT, reductions in fluorescence, and different patterns of IC amplification occurred. We suspected this was due to the 3M Integrated Cycler and/or IC DNA. In accordance with the package insert, IC reagents were stored at −20 °C and, once thawed, were stored at 4 °C for no more than 30 days.

Fig. 1 shows how the lids of our two 3M Integrated Cyclers differ: cycler 1 (left) has a 2-mm gap at the opening, while cycler 2 (right) does not (white arrows). To test the hypothesis that IC CT variations were due to cycler differences, a single master mix was prepared with identical samples run simultaneously on cyclers 1 and 2. The positive control has similar CTs for *B. pertussis*/parapertussis on cycler 1 (25.8/29.2) and cycler 2 (25.6/28.6), but the IC is only detected on cycler 2 (CT 31.5) (Fig. 1). *B. pertussis*/parapertussis negative patient sample has a higher IC CT on cycler 1 (34.6) than cycler 2 (32.9), and the y-axis fluorescence is approximately 3 times lower on cycler 1 (Fig. 1). Reduced fluorescence is probably due to different cycler–primer interactions with the IC DNA.

*Nonstandard abbreviations: IC, internal control; CT, crossing threshold.*

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