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Interference of Cerebrospinal Fluid Total Protein Measurement by Povidone-Iodine Contamination

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Abstract

Background—A falsely high cerebrospinal fluid (CSF) total protein (TP) result measured by pyrogallol red (PGR) method was suspected to be caused by preparation of the collection site with povidone-iodine (PVP-iodine) solution.

Methods—CSF TP was evaluated for interference in samples with different final concentrations of PVP-iodine (up to 0.25% PVP and 0.025% iodine) or iodine alone (up to 0.025% iodine) using three methods: PGR, modified biuret and benzethonium chloride (BZTC). Interference exceeding $\pm 20\%$ of the baseline value is considered clinically significant according to the criterion defined by College of American Pathologists.

Results—There was a positive interference with the PGR method and a negative inference for the BZTC method in CSF samples spiked with PVP-iodine. The PVP-iodine (up to 0.25% PVP and 0.025% iodine) did not cause a clinically significant interference with the modified biuret method. PVP alone without iodine caused a positive interference with the PGR method but did not interfere with the modified biuret or the BZTC method. When the samples were spiked with iodine alone, none of the three methods was affected (change $< 20\%$) by iodine concentration up to 0.025%.

Conclusions—Contamination of CSF specimens with PVP-iodine can lead to interference with CSF TP measurements using PGR or BZTC methods.

Keywords

Cerebrospinal Fluid; Total Protein; Preanalytical Interference; Povidone; Iodine

1. Introduction

The measurement of cerebrospinal fluid (CSF) total protein (TP) is important in the diagnosis of meningitis and the detection of other inflammatory diseases affecting the

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central nervous system. The clinical chemistry laboratory was contacted by a clinician concerned about discrepant increased CSF TP concentration (417 mg/dl; reference interval 15–45 mg/dl). The first tube (tube 1) of the CSF collection was used for the TP analysis using the pyrogallol red (PGR) sodium molybdate method on Siemens Dimension Vista (Siemens Healthcare Diag.). The patient had a history of acute lymphocytic leukemia and had an Ommaya reservoir in situ. CSF cell count results were as follows: red blood cells $6/\text{mm}^3$ and white cell count $1/\text{mm}^3$. The CSF TP measurement was repeated on the same sample with similar results (405 mg/dl). By contrast, CSF TP was measured on the same instrument as 20 mg/dl using another CSF specimen from the patient (tube 2) taken at the same time. The CSF TP concentration of 20 mg/dl was consistent with the patient's clinical findings. It was noted that tube 1 had yellow staining within the tube although the specimen itself was relatively clear (Supplementary data, Fig. 1). The yellow staining was suspected to have come from the antiseptic Betadine (PVP-iodine) applied to the skin at the site of the lumbar puncture. The laboratory postulated an interference of PVP-iodine in CSF tube 1.

2. Materials and methods

2.1 Materials

Antiseptic solutions containing iodine only (Iodine Tincture USP: Iodine 2%, Sodium iodide 2.4%, alcohol 47%) or PVP-iodine (USP Prep Solution, Triad Group) were from a retail supplier. For the PVP-iodine solution, the active ingredient is 10% povidone-iodine, USP, equivalent to 1% available iodine; and the inactive ingredients are citric acid, glycerin, nonoxynol 8, sodium hydroxide, and purified water. PVP was from Sigma Aldrich.

2.2 Interference studies

Two pooled CSF samples, a normal sample (normal CSF TP concentration) and a high sample (high CSF TP concentration), were used to prepare samples with different final concentrations of PVP-iodine (up to 0.25% PVP and 0.025% iodine), PVP (up to 0.25%) or iodine (up to 0.025%). Due to a change of instrument during the process of this investigation, the Siemens Dimension Vista analyzer was no longer available in the authors' institution. Siemens Dimension Xpand, which uses the same PGR method as that on the Vista, was used instead. CSF TP was subsequently measured using the PGR method on Siemens Dimension Xpand (Siemens Healthcare Diag.), a modified biuret method at a reference laboratory and the BZTC method on Roche Cobas 6000 (Roche Diag.)

The PGR method is an end point method involving the reaction of protein in the sample with PGR sodium molybdate complex to form a bluish-purple colored complex, which absorbs at 600 nm. In the modified biuret method protein forms a complex with cupric ions, which results in the dissociation of the cupric ion from a copper-azo dye complex. The decrease of the copper-azo dye complex is measured spectrophotometrically at 670 nm and is proportional to the concentration of protein in the samples. In the BZTC method the sample is preincubated in an alkaline solution containing EDTA. BZTC is then added producing turbidity, which is measured spectrophotometrically at 505 nm and is proportional to the concentration of protein in the sample.

2.3 Absorbance spectrophotometry

Absorbance spectra were obtained with a single-beam spectrophotometer (Cary 50, Varian) equipped with a 75-W pulsed xenon lamp, 2-nm fixed band-pass, and 24 000 nm/min maximum scan rate.

2.4 Statistical analysis

Interference exceeding $\pm 20\%$ of the baseline value is considered clinically significant according to the criterion defined by College of American Pathologists [1]. Statistical analyses were performed using GraphPad Prism version 6 (GraphPad Software).

3. Results

3.1 Interference

Fig. 1A-C show the effect of PVP-iodine, PVP and iodine, respectively, on the CSF TP measurement using PGR, modified biuret and BZTC methods. The range of CSF TP concentrations of non-spiked CSF samples measured by the three methods was 24–31 mg/dl (the normal sample) and 91–110 mg/dl (the high sample). CSF samples appeared clear on visual inspection (supplementary data, Fig. 2) when spiked with different final concentrations of PVP-iodine (up to 0.25% PVP and 0.025% iodine), PVP (final concentration up to 0.25%) or iodine (up to 0.025% iodine). Results for the PGR method showed a clinically significant ($>20\%$) positive interference by PVP-iodine or PVP alone (as low as 0.015% in the low sample). There was no clinically significant negative interference with the BZTC method until the PVP-iodine concentration reached 0.125% in the low sample and 0.25% in the high sample. In contrast, PVP alone (up to 0.25%) did not interfere with the BZTC method. Neither PVP-iodine nor PVP had a clinically significant interference in the modified biuret method. Iodine concentration up to 0.025% did not produce a clinically significant interference with any of the three methods.

3.3 Absorbance spectrophotometry

Spectrophotometric analysis of the patient's initial CSF sample and the PVP-iodine solution (without CSP) did not show an absorbance peak at 600 nm, 670 nm or 505 nm (data not shown), the wavelengths at which measurement occurs for the PGR, modified biuret, and BZCT methods, respectively.

4. Discussion

The majority of laboratory errors occur in the pre-analytical phase [2]. Correct collection of specimens is required in order to reduce errors in the pre-analytical phase. Important aspects of specimen collection include tube type and preparation of the patient/site for specimen collection. A prior study that examined the effect of PVP-iodine solution to clean venipuncture sites demonstrated significant interference with the following serum assays: phosphate, uric acid and potassium [3]. While PVP-iodine solutions are rarely used currently to clean venipuncture sites, they are commonly used as a skin disinfectant for other procedures including lumbar puncture. To the authors' knowledge this is the first study

describing interference of PVP-iodine solution in the PGR and the BZTC methods for CSF TP measurement.

In the case that we have described, the CSF sample had a slight yellow color, which was attributed to the presence of the iodine containing solution. Clinically significant interference of PVP-iodine in the PGR and BZTC methods was also detected in macroscopically clear samples. The interference of PVP-iodine was not due to direct spectrophotometric absorption since the PVP-iodine solution did not show an absorbance peak at 60 or 505 nm, the wavelengths at which the PGR and BZCT methods, respectively, are measured. The exact mechanism of PVP-iodine interference is unknown.

5. Conclusion

We recommend that solutions containing PVP-iodine should not be used for skin disinfection for any procedure where a needle is used to puncture the skin to collect a CSF sample for TP measurement using the PGR or BZTC methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations

BZTC	benzethonium chloride
PGR	pyrogallol red
PVP	povidone

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Highlights

1. PVP-iodine can cause interference with CSF TP measurements
2. Preanalytical errors must be considered when results don't match clinical findings
3. PVP-iodine must be used with caution for preparation of sites for lumbar puncture

a

b

c

Fig. 1.

Effect of PVP-iodine (A), PVP (B) or iodine (C) on CSF TP measurement using PGR (circle), modified biuret (square) or BZTC (triangle) methods. Two pooled CSF samples, a normal sample (solid line) and a high sample (dashed line), were used to prepare samples with different final concentrations of PVP-iodine (up to 0.25% PVP and 0.025% iodine), PVP (up to 0.25%) or iodine (up to 0.025%).