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Critical evaluation of ninhydrin for monitoring surgical instrument decontamination

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SUMMARY

Background: New Department of Health (England) Choice Framework for Local Policies and Procedures guidance (CFPP 0101) still states that ninhydrin can be used to check for efficient protein removal from surgical instruments processed in sterile services departments (SSDs).

Aim: With the potential transfer of variant Creutzfeldt–Jakob disease (vCJD) via surgical procedures it is necessary to re-evaluate recommended methods for protein detection.

Methods: This paper reports studies on the sensitivity and applicability of ninhydrin for detecting proteins in laboratories and SSDs. The efficiency of protein removal by swabbing was also evaluated.

Findings: Ninhydrin showed poor sensitivity toward proteins. Limits of detection for bovine serum albumin (BSA) in solution were 205 μg/mL compared with arginine 6 μg/mL. A commercial kit could detect neither rat brain homogenate nor BSA at <1000 μg protein pipetted directly into the vials. Swabbing with water-wetted rayon swabs was inefficient at removing protein (50 μg) from instruments ($N = 6$) with 32 ± 4% BSA and 61 ± 5% fibrinogen remaining bound. Swabs dipped in 0.5% detergent (Triton X-100) solution had slightly better removal efficiency with 20 ± 3% BSA and 24 ± 2.8% fibrinogen remaining.

Conclusions: Ninhydrin kits, currently used in SSDs, are ineffective at detecting residual proteins due not only to the insensitivity of ninhydrin towards proteins but also to the poor desorption of adhered proteins by swabbing. Overall ninhydrin, either as a laboratory reagent or as supplied in protein detection kits, does not provide sensitive detection of proteins and generates high numbers of false negatives when used in decontamination practices.

Introduction

Protein measurement is essential in many biological, clinical, food and forensic environments. Many common methods rely on reactions with the free amino groups within proteins that give coloured or fluorescent products. These visible or detectable reactions are used in determining proteins both qualitatively and quantitatively.[1–3]

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Residual proteins on re-useable surgical instruments, since they may include prions, may be directly related to the potential risk of transfer of variant Creutzfeldt–Jakob disease (vCJD) via instruments used in high-risk neurosurgical and posterior ophthalmic procedures. The Department of Health (England) risk assessments for vCJD transmission use the concept of total protein since suitably sensitive assays for prion proteins are not available. Following reports of inefficient decontamination of re-useable surgical instruments with high levels of protein measured post-decontamination in sterile services departments (SSDs), there was a concern that at that time failings of quality control and quality assurance procedures were occurring.[4–6]

The ninhydrin test is routinely employed by SSDs for residual protein detection on re-usable surgical instruments as specified in BS EN ISO 15883 Part 1 and the now archived Health Technical Memoranda (HTM) 0101/2030.[7] They include a reagent formulation, that is included in new DH (England) guidelines (CFPP 0101) part D.[8] Ninhydrin is used in many bioanalytical techniques, e.g. amino acid analysis.[9] Ninhydrin reacts with the primary amino groups of amino acids, forming an intensely coloured product known as Ruhemann's purple, after its discoverer, who observed that it stained skin purple.[10] It was later proposed and proved that the colour was due to a reaction with the free amino acids and ammonia in sweat.[11,12] Ruhemann's purple has a spectral maximum at 570 nm.

The intensity of the colour developed in the reaction depends on the volume, number and chemical nature of the amino groups being analysed. Studies have suggested that the reaction mechanism involves displacing the hydroxyl group on ninhydrin hydrate by a primary amino group with the formation of a Schiff base.[13] Alkaline pH promotes reduction of ninhydrin to hydrindantin, thereby enhancing colour formation.[14] Moore and Stein determined the optimum pH for the overall reaction to be 5.5.[15]

The technique outlined in CFPP 0101 to assess instrument cleanliness requires swabbing of the instrument surface with a water-wetted swab followed by reacting the swab tip with the specified ninhydrin solution. Detection is either visual or in a simple colorimeter using the amino acid, arginine, as the positive control.

The present study was carried out to investigate: (i) the ability of the ninhydrin method to detect intact protein as opposed to free amino acids and thereby assess its applicability in the detection of low levels of residual proteins on surgical instruments; (ii) the efficiency of wet swabbing as a protein removal method from surgical instrument surfaces.

Methods

Proteins, amino acids, ninhydrin, Triton-X-100, *o*-phthaldialdehyde and *N*-acetyl-cysteine were from Sigma (Poole, UK). Decon 90 was from Decon Laboratories (Brighton, UK). Rayon swabs with plastic handles were from Sterilin (Caerphilly, UK). Ninhydrin protein detection kit (no. 2370) was from Albert Browne (Leicester, UK). Bradford reagent protein assay kit was from Bio-Rad Ltd (Hemel Hempstead, UK).

Qualitative studies on protein detection using ninhydrin

The studies were performed using reagent prepared according to Moore and Stein's optimized formulation.[15] Ninhydrin (178 mg) was dissolved in 10 mL methanol and then diluted to 100 mL with 10 mM aqueous sodium acetate buffer (pH 5.5). The solution was stored at 4 °C prior to use. Standard solutions (1 mg/mL) of L-leucine, L-arginine, bovine serum albumin (BSA), human serum albumin, globulin, fibrinogen, haemoglobin, myoglobin and cytochrome-*c* were prepared in deionized water. Human plasma and whole blood were also tested. Of each solution, 25 μL were pipetted directly on to rayon swab tips that were immersed in ninhydrin reagent (500 μL) and heated at 60 °C for 30 min. The swabs were removed and photographed with a digital camera (DSLR-A380, Sony) against a white background for visual confirmation of the purple colour formation.

Qualitative studies on protein detection using a commercial ninhydrin kit

BSA solutions (0.1–10 mg/mL) were prepared and 100 μL of each was pipetted directly into the reaction tubes containing ninhydrin reagent as supplied in the ninhydrin protein detection kit. The vials were capped and incubated at 60 °C for 60 min, as per the manufacturer's guidelines. The tubes were photographed as above to record colour changes. The positive control was 50 μg arginine and the negative control was a swab wetted with deionized water.

Quantitative studies with ninhydrin

Standard solutions (0.02–0.1 mg/mL) of alanine, leucine, arginine and lysine were prepared in deionized water. BSA, fibrinogen, β-casein, lysozyme and γ-globulin standard solutions (0.2–1 mg/mL) were prepared in deionized water. Each dilution (300 μL) was mixed with 1 mL 0.3% w/v ninhydrin in 70% iso-propanol as per CFPP 0101. The mixture was heated in boiling water for 30 min in sealed tubes. The absorbance was measured at 570 nm in a spectrophotometer (model 6705; Jenway, UK). The repeatability of the method was evaluated using 10 replicate standards of arginine and BSA. The experiment was repeated using the Moore and Stein formulation described above.

Protein removal from surgical steel by swabbing

Bovine serum albumin (50 μg) and fibrinogen (50 μg) in water were pipetted (20 μL) on to three separate, but similar, surgical instruments and dried at ambient temperature for 24 h. For each contaminated set of instruments the first instrument was not swabbed, the second was swabbed with a rayon swab dipped in deionized water and the third with a swab dipped in a non-ionic detergent solution (aqueous 0.5% Triton X-100). Each instrument was then placed in a 50 mL polypropylene tube filled with 0.1% Decon-90 solution to cover the area contaminated and/or swabbed and sonicated for 1 h in a QS3 ultrasonic bath (Ultrawave, Cardiff, UK). After removing the instrument the wash volume was recorded. The protein concentration in each wash ($N = 3$) was determined using a fluorescence method based on *o*-phthaldialdehyde/*N*-acetyl-cysteine previously described by Smith *et al.*[16] The total protein on the instrument was calculated using the wash volume.

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