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## ORIGINAL ARTICLE

# Biotin Interference in Susceptible CanAg Ca242 Immunoassays and Elimination Method

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### SUMMARY

***Background:*** **High-dose biotin therapy is beneficial in progressive multiple sclerosis (MS). Biotin, as dietary supplement or therapy, may lead to analytical interference in biotin-streptavidin immunoassay.**
***Methods:*** **Seven concentration gradients of biotin solutions were spiked to three different levels of Ca242 serum samples. All samples were tested by CanAg Ca242 ELISA kit to evaluate the interference from biotin. Serum samples with biotin concentration at 1,000 ng/mL were retested after absorption by streptavidin microparticles or direct analysis on the Mindray CL2000i platform.**
***Results:*** **Our study found that CanAg Ca242 is vulnerable to interference when a sample that contains biotin exceeds 15.63 ng/mL. Biotin interference can result in falsely low results in CanAg Ca242. The effect and extent of biotin interference are, to some extent, dependent on the concentration of serum Ca242 and the concentration of biotin.**
***Conclusions:*** **CanAg Ca242 is vulnerable to biotin interference. The laboratory can overcome biotin interference on CanAg Ca242 by using a non-biotin streptavidin method or by absorbing biotin with streptavidin-coated microparticles before testing. Clinicians should use caution in interpreting abnormal results in patients who ingest biotin.**
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### KEY WORDS

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### INTRODUCTION

Biotin, also known as vitamin B7, is a water-soluble vitamin found in the regular diet such as egg yolk, pork, soybeans, and vegetables [1]. Like other members of the vitamin B complex, biotin is water-soluble and not toxic. The vitamin B complex usually contains approximately 30 μg biotin [2]. Oral biotin is almost 100% bioavailable if given as a supplement and also in pharmaceutical doses [3]. It is reported that in nail diseases receiving 2.5 - 5 mg/day will improve the symptoms [4]. A review showed 18 reported randomized clinical trials of biotin use for hair and nail changes, all cases that received biotin supplementation showed evidence of clini-

cal improvement [5]. Children with inherited biotinidase deficiency are routinely treated with 5 - 20 mg of biotin per day independent of their age [6]. A pilot study showed that in progressive multiple sclerosis, receiving the biotin dose from 100 to 600 mg/day (median = 300 mg/day) might have an impact on disability and progression in progressive multiple sclerosis [7]. Biotin intake is quite common in humans that brings some challenges to the streptavidin/biotin-based immunoassays, which are widely used in routine clinical laboratory. Because biotin in the sample will compete with biotinylated reagents for the binding sites on the streptavidin reagents [8-10], it was reported that exogenous biotin interferes with assays on the Elecsys platform, Siemens Dimension Vista 1500, Siemens Centaur et al., which are based on the biotin-streptavidin immunoassay [11-15].

Ca242 first was isolated in 1985 by Lindholm et al. It is a new tumor marker for pancreatic cancer [16]. Preliminary studies have shown that Ca242 is a useful complement for pancreatic cancer and colorectal cancer [17, 18]. CanAg Ca242 is also a biotin-streptavidin based sandwich EIA. The analyte is sandwiched between the signal antibody and the biotinylated antibody, which links the antibody-analyte sandwich to a streptavidin-coated solid phase. If no interference is present, the assay signal is directly proportional to the analyte concentration. However, when excess biotin is present in the specimen, the biotin molecules will saturate the streptavidin-binding sites, thus preventing the antibody-analyte sandwich from binding with the streptavidin-coated solid phase to generate assay signal after the washing phase. Hence, in the presence of high biotin concentrations, the intensity of the assay signal would decrease, which results in an underestimation of the actual analyte concentration, thus causing harmful interference [8]. Based on the previous studies, we hypothesize that serum biotin can cause interference with CanAg Ca242 sandwich assay. Our study will investigate the vulnerability of CanAg Ca242 to exogenous biotin, and the effect of analyte concentration on biotin interference, and then find a better way to solve the biotin interference.

## MATERIALS AND METHODS

### Preparation of biotin samples

Pure biotin was dissolved in 0.01 M NaOH and stored at -20°C for preservation. Biotin stock was diluted with PBS into working solutions with nine concentration gradients that were then spiked into three different baseline Ca242 samples to achieve the indicated final concentration of biotin (1,000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81 ng/mL). For controls, serum samples were spiked with an equal volume of PBS to account for possible matrix effects. The volume of the spike was 10% of the final volume.

### Biotin neutralization method

The samples were absorbed with magnetic microparticles coated with streptavidin. This reagent was included in the Cobas® assay kits supplied by Roche, and the streptavidin concentration is 0.72 mg/mL. The microparticles were isolated from Roche M reagent solution, then distributed to the polystyrene tubes and let air dry in a 4°C refrigerator so that they would not dilute the specimen. Samples with biotin concentration at 1,000 ng/mL were added to streptavidin microparticle tubes, and the final concentration of streptavidin microparticles in all spiked sera samples were 21.6 mg/mL. The streptavidin microparticle tubes were incubated with serum samples and left for one hour shaking at room temperature (300 rpm), then centrifuged for 20 minutes at 3,000 rpm/minute, then reanalyzed.

### Measurement of Ca242

Serum samples were measured on the CanAg Ca242 ELISA kit, which is based on the biotin-streptavidin immunometric sandwich assay.

### Statistical analysis

Statistical comparison between biotin-spiked samples and PBS solution-spiked baseline samples was performed by GraphPad Prism 7. Line charts were used to show the interference effect.

The relative deviation in a test result > 10% between the baseline sample and the biotin-spiked sample was considered significantly different.

## RESULTS

### Biotin interference at different baseline values of Ca242

Data showed three different concentrations across the analysis of measurement ranges of Ca242 had significant interference from biotin, but the magnitude of the interference was variable at different concentrations of biotin. The higher the biotin concentration, the more significant the reduction of Ca242 (Figure 1).

Our data demonstrate that the initial biotin interference effect depends on the concentration of Ca242 (Table 1). The relative deviation between the baseline sample and the biotin-spiked samples were compared, in order to evaluate the interference effects on different concentrations of Ca242 at various concentration gradients of biotin (Table 1). A relative deviation greater than 10% was considered a significant difference according to the internal quality control target. The Ca242-low level, cutoff level, and high level showed significant differences respectively at biotin concentrations of 15.63 ng/mL, 31.25 ng/mL, and 62.5 ng/mL. At the biotin concentration of 250 ng/mL, the relative deviation was more than 99%.

**Table 1. Relative deviations between Ca242 baseline samples and biotin-spiked samples at different biotin concentration.**

| Biotin (ng/mL) | Relative deviation | | |
|---|---|---|---|
| | % Low level | % Cutoff level | % High level |
| 7.81 | 9.91 | 3.77 | 1.01 |
| 15.63 | 16.05 * | 3.95 | 2.68 |
| 31.25 | 31.91 | 11.11 * | 5.03 |
| 62.5 | 78.18 | 61.02 | 39.46 * |
| 125 | 99.10 | 98.90 | 73.69 |
| 250 – 1,000 | > 99.9 | > 99.9 | 88.49 - 99.3 |

* - relative deviation between biotin-spiked sample and unspiked baseline sample > 10% is a significant difference.

**Table 2. Biotin-spiked Ca242 samples before and after biotin neutralization.**

| Samples | Ca242 concentration | | |
|---|---|---|---|
| | Low level | Cutoff level | High level |
| Baseline PBS-spiked samples | 11.25 | 26.55 | 149.86 |
| Biotin-spiked samples (Biotin: 1,000 ng/mL) | 0.001 | 0.002 | 0.105 |
| Neutralized biotin samples | 10.30 | 26.40 | 149.00 |

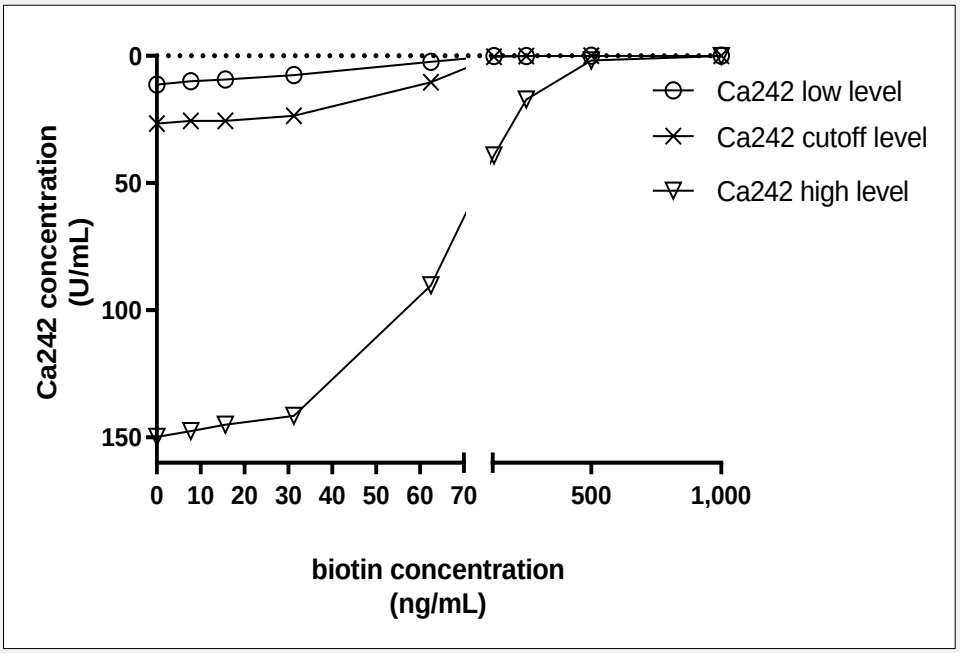


**Figure 1. The effects of increasing concentrations of biotin on the results of Ca242 at three different baseline analyte levels.**

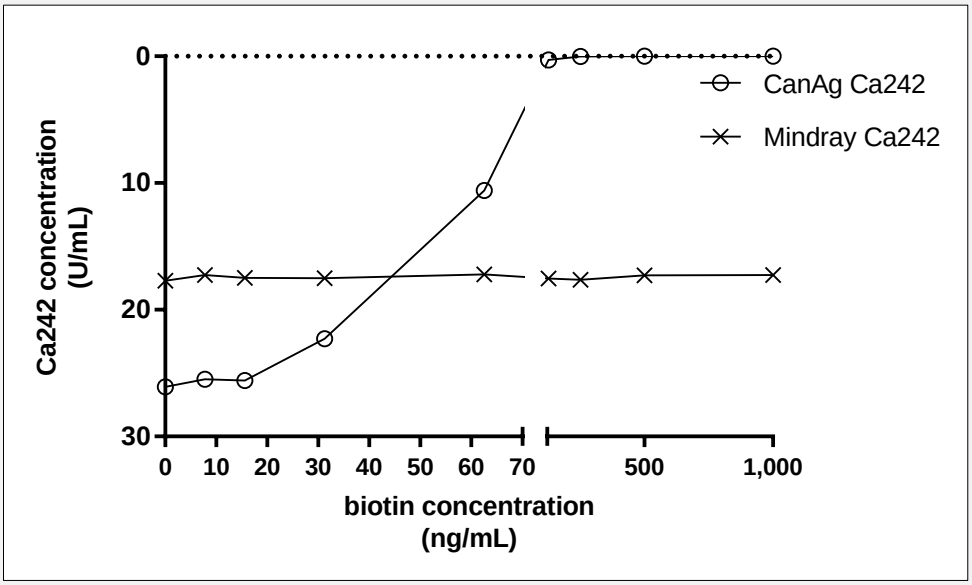


**Figure. 2. Biotin effect on CanAg Ca242 and Mindray Ca242 platforms.**

**Biotin interference could be neutralized by streptavidin**

After absorbing with streptavidin-coated microparticles, all samples with spiked-biotin concentration at 1,000 ng/mL had recovered close to the baseline levels (Table 2).

**Biotin interference does not exist on the non-biotin/ streptavidin platform.**

A set of biotin spiked samples were tested both on CanAg Ca242 ELISA kit and Mindray CS2000i analyzer. The biotin interference in CanAg Ca242 was dose-dependent, whereas the biotin interference does not exist in Mindray CS2000i platform, which uses a magnetic bead-based capture system instead of biotin-streptavidin linkage.

## DISCUSSION

Biotin interference in routine clinical immunoassays has been increasingly reported [11-15,19-22], owing to the rising popularity of biotin as a supplement and its pharmacologic use for some diseases [23,24]. Usually, different streptavidin/biotin detection platforms have different susceptibility to biotin interference. However, most of the studies [11,20,22,25], including ours, are based on the addition of exogenous biotin to the serum. It can only reflect the interference effect of biotin in human body indirectly. HPLC/avidin binding assay is the reference method to analyze biotin. But, only a few reference laboratories can offer and this method may be inappropriate for measuring high concentrations of serum biotin [10,26]. Simpler and cheaper methods for measuring serum biotin are currently lacking in labs. Nevertheless, the lab and the clinician still can obtain a suitable warning from these interference studies.

It was reported that in the groups that received a single dose of 100 or 300 mg, peak serum biotin levels reached as high as 494.9 ± 161.0 ng/mL (at 1.25 hours) and 823.8 ± 303.1 ng/mL (at 1.5 hours), respectively [27]. Five healthy adults consumed 1 mg of biotin daily for seven days, producing a mean biotin plasma level of 34,600 ± 8,000 pmol/L in blood samples drawn 1 - 3 hours after the last biotin dose [28].

Our study found that CanAg Ca242 is vulnerable to interference when a sample contains biotin exceeding 15.63 ng/mL. Biotin interference can result in falsely low results in CanAg Ca242. The effect and extent of biotin interference are, to some extent, dependent on the concentration of serum Ca242 and the concentration of biotin (Figure 1). The data of Table 2 show that the Ca242 low value is more susceptible to biotin interference than high value. Moreover, 62.5 ng/mL serum biotin produces a gross reduction in all analytical concentration. When biotin concentration was higher than 250 ng/mL, the relative deviation was more than 99.9% in all samples, which lead to opposite erroneous results, as urinary excretion is the primary route of biotin clearance, and the interference is most likely amplified in

chronic kidney disease [25,26].

In general, patients taking over the counter biotin up to 5 mg/day should be advised to withhold the supplement for at least 8 hours preceding their blood tests if clinically possible which should be sufficient time to allow for biotin clearance and removal of the majority of assay interference independent of the manufacturer, with the exception of patients taking biotin doses up to 100 - 300 mg/d for whom it is recommended to wait at least three days [3,27]. However, for individuals with renal failure biotin may cause interference for up to fifteen days after the last dose [29]. Sometimes, the biotin intake or the serum concentration is not known precisely; under these circumstances, our study can provide a workable way to remove interference. Based on the strong affinity to streptavidin, we used streptavidin microparticles to "pre-bind" biotin. After pretreatment, the results had recovered firmly to the baseline levels (Table 2). As a comparison, we performed parallel experiments on the Mindray CL2000i. The data shows that biotin interference does not appear in the Mindray CL2000i intact Ca242 assay, which does not use the streptavidin/biotin method (Figure 2).

So, if the clinician questioned the unreasonable or contradictory results, the laboratory should be aware of the current analytical methodology and possible interference. The patient questioned regarding his biotin intake to estimate the potential and magnitude of biotin interference. In our lab, we found existing interference for CanAg Ca242 at the initial biotin concentration of 15.63 ng/mL. We recommend the non-biotin avidin detection platform, if available, or retest after neutralizing the biotin.

## CONCLUSION

In summary, our data can be used by laboratories to set surveillance thresholds to identify biotin interference on CanAg Ca242 immunoassay. In addition, we provide a reference to solve the biotin interference in immunoassays.

**Declaration of Interest:**

The authors declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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