age and sex, the most influential factors affecting serum ferritin concentrations were blood donation, followed by etha-

nol intake in men and diet in women. Interestingly, these authors measured ferritin in two large asymptomatic Aus-

tralian groups (n = 1367 and 601) and concluded that iron stores were more than twice as high as previously re-

ported and that reference ranges for fer-

ritin in the Australian population should be significantly increased.

Our data indicate that, in addition to age and sex, laboratories should consider documenting the degree of

blood donation and iron supplementation

within their population when estab-

lishing reference ranges for ferritin and other iron-related data.

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Platelet-Poor Plasma Not Suitable

for Clinical Endotoxin Testing,

Demonstrated in Horses

To the Editor:

Endotoxins are the lipopolysaccha-

ride (LPS) constituents of the outer

cell wall of Gram-negative bacteria.

They initiate clinical phenomena asso-

iated with septicemia by triggering

synthesis of mediators such as tumor

necrosis factor (TNF) and interleukin

1 (IL-1), IL-6, and IL-8. Therapeutic

clinical trials have been performed in

septic patients with monoclonal anti-

bodies to LPS or TNF, or soluble IL-1

receptor antagonists (1-4). In one study,

patients with detectable LPS in their

circulation benefited most from ad-

ministration of anti-LPS antibodies

(5). LPS was not measured in the

other studies.

In our opinion, LPS measurements

are essential for selecting which pa-

tients will be most likely to benefit

from anti-LPS, anti-TNF, and other

treatment strategies. However, reli-

able quantification is imperative. Clin-

ical LPS testing can be hampered by

environmental contamination during

blood collection and handling proce-

dures, providing false-negative results.

Conversely, the possible removal of

LPS by cellular binding and cell re-

moval during the blood handling would

provide false-negative results. Specifi-

cally, LPS binding to platelets is con-

troversial. Experiments with LPS-

supplemented human whole blood

provided similar LPS concentrations in

platelet-rich plasma (PRP) and plate-

let-poor plasma (PPP) (6, 7), but

binding to platelets in human blood in

vivo has been reported (8-11). Re-

cently, Redi et al. (12) reported the

usefulness of a commercially available

LPS-free blood collection tube for

LPS and cytokine testing (Endo Tube

ET®, Chromogenix, Möln达尔, Sweden).

The recommended use of the tube pro-

vides PPP (centrifugation at 3000g gen-

erates an intermediate gel layer for op-

timal separation of cells and plasma),

which may result in substantially less

LPS being detected in clinical sam-

ples.

From 48 horses with naturally oc-

curring acute intestinal disorders, we

collected by aseptic venipuncture of

the jugular vein duplicate 4-ml blood

samples for the possible assay into

Endo Tube ET containing 120 IU of

sodium heparin. Blood was obtained

just before anesthetization, after ap-

parent restoration of bowel perfusion (as

estimated by color and motility in the

segments bordering the affected bowel

part and the part itself), and every 8 h

thereafter for a 48-h follow-up period.

The samples were immediately

mixed in heparin, and within 20

min centrifuged at 4°C either at 170g

for 15 min to obtain PRP or at 2200g

for 15 min to obtain PPP. We then

removed the plasma layers and stored

each in two aliquots at -23°C. Platelet

counts were performed in PRP of

EDTA-anticoagulated blood samples

drawn at the same collection times

and sites during the study periods,

with a Sysmex K-1000 cell counter

(Toa Medical Electronics, Kobe, Ja-

pan). After 10-fold dilution in LPS-

free water and heating for 15 min at

75°C to remove plasma inhibitory

activity, LPS was measured with a chro-

mogenic Limulus assay (Coatest®, En-

dotoxin; Chromogenix) as described

elsewhere (7). The assay has a detec-

tion limit of 3 ng/L in PRP, as deter-

mined by the LPS concentration cor-

responding to the mean +3 SD

absorbance (A400) of the blank mea-

sured 10 consecutive times. The cli-

cinal decision limit for the presence or

absence of endotoxemia, i.e., endo-

toxin positivity, was arbitrarily set at

LPS = 5 ng/L PRP, similar to the de-

cision limit for humans (13), given the

absence of studies to establish this

limit in horses. For our study, 12

healthy horses, LPS concentrations in

PRP never exceeded 4 ng/L (data not

shown).

The blood collection tube proved to

be convenient and suitable: Only 8

samples of 307 (145 concurrently

prepared PRP and PPP and 177 PPP)

were thought to be contaminated dur-

ing blood collection or further han-

dling on the basis of marked differ-

ences (>100 ng/L) between LPS con-

centrations in the paired PRP and

PPP samples. Of the PRP samples, 38

were LPS-positive (mean 19.8, range

5-197 ng/L). However, the LPS con-

centrations in PPP were significantly

higher (P = 0.008, Wilcoxon signed

test rank) than in the concurrently

prepared PPP (mean 10.9, range 3-

145 ng/L). The lower LPS concentra-

tions in the PPP samples meant that 7

of the 38 LPS-positive PRP samples

were graded as negative (LPS < 5 ng/

L). The percentage difference (ex-

pressed as % binding of LPS to plate-

lets) between the LPS concentrations

from these samples was correlated

with the platelet counts in the PRP

samples (r = 0.53, P = 0.001, n = 38,

range 8-77%, Fig. 1). This weak but

highly significant correlation suggests

that binding of LPS to platelets may

be the cause of LPS loss during prepa-

ration of PPP. This hypothesis needs

further investigation.

We conclude that the Endo Tube ET
is a useful tool for collecting blood for LPS testing in clinical situations, but that PRP must be used instead of PPP to avoid inadvertent removal of LPS. The gel layer in the tube may be useful for providing optimal separation between cells and plasma for cytokine studies, and it does not interfere with LPS measurements.

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should Accuracy of Iron Measurements Be Improved?

To the Editor:
An editorial by Eckfeldt and Witte (1), published in connection with our article on the status of serum iron measurements (2), questions whether or not analytical improvement of serum iron measurements will enhance the outcome for the patient. The authors raise several issues that warrant extensive discussion and require ultimate resolution. Unfortunately, they also raise arguments and make statements that confuse rather than clarify the issues, especially some important issues presented in our publication.

The apparent strong opposition of the authors to serum iron measure-

ments is somewhat puzzling, especially in view of their statements that:
1. The real question is whether improving serum iron methods analytically will substantially improve the clinical utility of such measurements. The authors thereby imply that the jury is still out on this issue.
2. "...all laboratory approaches for assessment of iron deficiency have limitations in specific patients." The authors thereby imply that iron measurements would be useful in at least some patients. This is especially true if these determinations are accompanied by other carefully selected tests such as total iron-binding capacity (TIBC), transferrin, or ferritin assays. Eckfeldt and Witte themselves point out that release of iron from the reticulendothelial system in patients with renal failure is inadequate (functional iron deficiency), while serum ferritin values are high in these clinical situations; thus, iron measurements should provide additional information regarding iron release. In addition, measurements of serum iron and transferrin saturation in conditions of iron overload are well-established diagnostic tests with proven clinical utility.
3. There has been "a recent upsurge in clinical interest in iron measurements." In fact, >3500 laboratories participated in a recent survey of the College of American Pathologists (CAP) (3). In most of these laboratories the determination of iron is a relatively high-volume test. In contrast, serum ferritin measurements are performed in only about two-thirds of laboratories participating in the CAP survey.

In light of these facts, how can we "call off the hounds" and abolish our efforts to improve the analytical determinations of serum iron? As long as these iron assays continue to be in such wide use and are clinically helpful in at least some clinical situations, patients deserve that these tests be performed as well as the state of the art reasonably allows. A television commercial comes to mind: "If we cannot do it right, we don't do it at all." Which shall it be?

Eckfeldt and Witte also state that copper is a major interferent and that hemolysis contributes to increased serum iron values. Not so! When methodologies are properly selected, these two factors will not substantially affect iron values. These problems exist only if inferior methodologies (regardless of how convenient they may be) continue in use.

In their editorial, the authors show little enthusiasm for the development