ZOETIS DIAGNOSTICS



Dilution Guide

This guide provides information and resources on sample dilution for the Vetscan Imagyst[®] Al Urine Sediment Application.



For additional support, contact:

(888) 963-8471 (option 5) dxsupport@zoetis.com

Need guidance on a treatment plan?

Confirm results and a path forward for complex cases with remote specialist consultations at no additional charge for Zoetis Diagnostics customers.*

* Requires the use of ZoetisDx.com, the Vetscan[®] Fuse and at least one Zoetis Diagnostics instrument or service.

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What is Dilution?

Dilution is the action of making the urine sample more dilute, in order to evaluate a less concentrated urine sediment sample (Figure 1). This process facilitates element identification as it ensures formed elements are adequately spaced with no overlap.

When and Why to Dilute?

It is important to evaluate the color and clarity of the urine sample to determine if dilution is necessary (See Table 2).

Be sure you're using a clear specimen container to evaluate urine color and clarity.

Several factors can create abnormal urine color, such as metabolic or pathological conditions, muscle damage, or drug intake. Turbidity can occur due to crystals, cells, mucus, fat, bacteria, casts, and potentially spermatozoa in the urine.

A urine sediment evaluation is essential to determine which formed elements are contributing to the color and clarity of the urine sample.

Figure 1 Pre- and Post-Dilution (1:8) of a Hematuric Sample (40x)





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Gross hematuria, when blood is visible in urine, is the most common reason to perform a dilution. The pre- and postcentrifugation color guide provided (Figure 2) can help you determine if a dilution should be performed prior to scanning the urine sediment sample.





The intensity of orange-red color observed will inform the dilution ratio. While the guide provides suggested dilution ratios, a veterinary professional will need to determine the appropriate dilution for a given sample.

Figure 2 Suggested Dilution Ratios Based on the Color of the Urine Sample



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8 Steps to Dilution

Figure 3 8 Steps to Dilution Using the Vetscan Imagyst If dilution of the sample is needed, the following flowchart provides a step by step guide to the the process.

non-pathologic crystal formation.





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As Figure 3 on the previous page outlines, centrifuge the sample and then drain the supernatant. Choose an appropriate dilution ratio based on color (Figure 2). Then, add the appropriate amount of sterile saline to reach the new desired concentration (Table 1). Finally, gently re-suspend pellet in the supernatant/saline mixture, place 20µl of the well-mixed sample on the slide, and scan immediately (Figure 4).

Table 1 Dilution Preparation Add sterile saline (0.9% NaCl) to residual/concentrated urine to reach the desired dilution ratio.

| Dilution Ratio (Approximate) | Residual Concentrated Urine Volume* | Sterile Saline Volume (0.9% NaCl)* | Corresponding sample preparc |
|---------------------------------|--|---------------------------------------|---------------------------------|
| 1:2 | 0.35 ml | 0.65 ml | 1 ml |
| 1:5 | 0.35 ml | 1.65 ml | 2 ml |
| 1:8 | 0.35 ml | 2.65 ml | 3 ml |

Figure 4 Dilution Process

Centrifuge, decant supernatant, and add the appropriate saline volume. Note that the correct amount of saline will fill the tube to the 1 ml, 2 ml, or 3 ml lines.



Centrifuged Sample







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The color of the supernatant after centrifugation is useful to determine the need for dilution. If at step 3 of Figure 3 the supernatant is pink, red, or brown, the sample may be showing signs of hemoglobinuria or myoglobinuria (Table 2). If this is the case, process the sample without dilution.

Alternatively, if at **step 3** the supernatant clears, the sample is likely red or cloudy due to formed elements like RBCs and/or WBCs. In this instance, dilution is probably necessary.

Table 2 Interpretation of Pre- and Post-Centrifugation Urine Color

Color Pre-centrifugation

Color Post-centrifugation

RBC present in Urine Sedim

Plasma Color

Other Evidence



| | Hematuria | Hemoglobinuria | Myoglobinuria |
|-------|--|----------------|---------------|
| | Red, Brown, Pink 🛛 🔴 🔴 | Red, Brown 🔴 🔴 | Red, Brown |
| | Straw/Yellow – – | Red, Brown 🔴 🔴 | Red, Brown |
| ment? | Many | None to Few | None to Few |
| | Normal | Pink | Normal |
| | Urinary Tract Disease, Traumatic Urine Collection | Anemia | Muscle Damage |



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