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Technical Tip 06

ZEUS AtheNA Multi-Lyte® Test Systems

Subject: Frequently Asked Questions on the HEp-2 Bead

The HEp-2 Bead can be found in the bead suspension of the following AtheNA Multi-Lyte® ANA Test Systems: AtheNA Multi-Lyte® ANA II Plus (PN:A21101), AtheNA Multi-Lyte® ANA (PN: A20001) and AtheNA Multi-Lyte ANA III (PN: A22001).

1. **What is the purpose of the HEp-2 bead.**
   When using conventional ANA screening methods such as IFA ANA HEp-2, it is not uncommon to see specimens that are ANA screen positive and negative to all common reflex tests. If the AtheNA Multi-Lyte® ANA Test Systems did not have the HEp-2 bead, such samples would generate a negative result. By including the HEp-2 bead in the ANA bead suspensions, such samples display the same outcome; positive for ANA screen and negative for the reflex test. Therefore, the purpose of the bead is to more closely simulate a conventional ANA screen test, rather than a panel of reflex tests.

2. **Is the HEp-2 bead used as the ANA screening portion of the assay?**
   The AtheNA Multi-Lyte® ANA Test Systems have ten antigen-coated beads in their bead suspension, one of which is the HEp-2 bead. All ten beads contribute to the qualitative ANA screen outcome, not just the HEp-2 bead. If a patient is positive to one or more of the ten beads, then that patient is considered ANA positive.

3. **Why can't I see the results of the HEp-2 bead?**
   The HEp-2 bead is made by taking nuclei from HEp-2 cells and conjugating that material onto the surface of the bead. The bead will then theoretically contain numerous nuclear antigens, many of which may not be properly identified. However, it is known that some of the properly identified antigens do not conjugate well onto this bead and could be missed. For example, if the SSB antigen does not conjugate on to the surface of the HEp-2 bead, then a patient that should is positive for SSB would be missed. This is resolved in the AtheNA Multi-Lyte® Test Systems because there is a separate SSB bead in the bead suspension that is specific for of the detection of the SSB antibody. However, if the results for the HEp-2 bead would be shown for SSB positive patients, the HEp-2 bead would be negative and the SSB bead would be positive. It is likely that users would assume that the SSB bead was a false positive. For this reason the FDA mandated

the result of the HEp-2 bead not be shown on the results. However, the HEp-2 bead is still being used and is getting a result of its own, even though it's not being shown.

4. **Since I can't see the results of the HEp-2 bead, how do I know if it does contribute to the ANA screen result?**
   There is one very simple way to see that the HEp-2 bead is contributing to the qualitative ANA outcome. If all ten test results are requested for the specimens, occasionally there will be a patient that will be ANA positive but negative for the other nine markers. The only way that can happen is if the patient is positive to the HEp-2 bead. The frequency of these types of samples is very dependent upon the population of specimens in each laboratory. However, since AtheNA Multi-Lyte® Test Systems are designed to be much more clinically specific than most ANA IFA tests, you may not see these types of samples very frequently.

5. **If a patient is positive for the HEp-2 bead and negative for the other nine markers, what kind of autoantibody do they have?**
   The possibility is nearly endless. There are numerous rare, but fairly well known autoantibodies that are not captured by the other nine analytes, but are known to be detected using HEp-2 cells. Some examples of these autoantibodies include ssDNA, centromere (other than centromere B), fibrillarin, PMScl.

6. **If I quantify the qualitative ANA result, where does the number come from?**
   The AtheNA Multi-Lyte® Software gives you the option to report the qualitative ANA outcome with a numerical result. The number that appears for the qualitative ANA outcome is the largest number from all ten bead set. With the AtheNA Multi-Lyte® ANA Test Systems, all ten beads have the same interpretation criteria; greater than 120 AU/mL is positive, less than 100 AU/mL is negative and from 100 to 120 AU/mL is equivocal. Occasionally, one may see a patient that has a numerical result that is greater than any of the nine assays displayed. If that occurs, it is because the result on the HEp-2 bead was greater (numerically) than any of the other nine assays. This is not uncommon, and this is not indicative of any kind of a problem. It simply means that for that patient, very much of their antibody reacted to the HEp-2 bead. For example, if a person has 900 AU/mL of SSA, 600 AU/mL of Sm and 800 IU/mL of dsDNA, since all three of these autoantibodies may react with the HEp-2 bead, there is a chance that the result on that bead may be greater than any of the others.