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Application Note

Performance Comparison of Estapor® Red Intense Microspheres versus Colloidal Gold as Detector Particles in a FluA Lateral Flow Immunoassay

Summary

While lateral flow immunoassays are famous point-of-care diagnostic tools, they still face certain challenges, such as achieving higher sensitivity and ensuring reliable results. This report illustrates how Estapor® Red Intense microspheres can enhance the performance and reliability of lateral flow immunoassays compared to colloidal gold, with the following key advantages:

- Estapor® Red Intense microspheres outperform in sensitivity, detecting lower analyte concentrations with up to four times stronger test line intensity.
- Compared to colloidal gold, Estapor® Red Intense microspheres provide faster test line appearance, allowing for visualization up to five times quicker, particularly at low analyte concentrations.
- The precision of results using Estapor® Red Intense microspheres is comparable to results using colloidal gold, ensuring reliability and reproducibility.

Introduction

Lateral flow immunoassays (LFIA) (**Figure 1**) have emerged as a crucial diagnostic tool. Their simplicity, accessibility, and ability to deliver timely results make them a valuable tool for disease detection in a point-of-care setting, which contributes to improved patient outcomes and public health management.

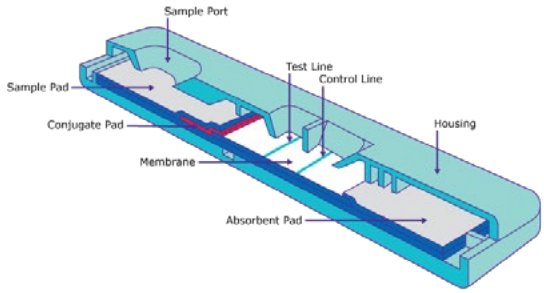

Figure 1 – Lateral flow immunoassay design.

While LFIA offer numerous advantages, they also face certain challenges that researchers and developers are actively working to address. Some of the current challenges include achieving higher sensitivity and lower limits of detection, ensuring specificity to minimize false results, developing multiplexing capabilities, maintaining stability and shelf-life, reducing user variability in interpretation, and streamlining assay development processes.

To address these challenges, we published a study showing that our innovative Estapor® Red Intense microspheres[1,2], in combination with our well-known Hi-Flow™ Plus membrane, are superior compared to other red polystyrene particles on the market[3]. The present report focuses on the performance comparison of Estapor® K1-030 Red Intense microspheres (**Cat number: FR180380637**) to colloidal gold particles in a LFIA application[4] (**Table 1**). As part of our journey to excellence, the technical application work was completed by our new Assay Development Services team[5].

Table 1: Characteristics of detector particles.

Product Name	K1-030 Red Int COOH PS 5 %	Gold Colloid: 40nm
Chemical nature	Polystyrene	Colloidal gold
Supplier	Merck	Supplier A
Functional group	COOH (200 – 500 µeq/g)	None
Diameter (range)	0.270 - 0.330 µm	0.037 – 0.043 µm
Concentration	5 % w/w	OD 1

Results and Discussion

As recently exemplified by the COVID-19 pandemic, infectious disease diagnosis is crucial for public health management[6]. Accurate and early diagnosis plays a vital role in improving patient outcomes and protecting public health. Test sensitivity is a critical measure of a diagnostic test's ability to accurately identify individuals with the target analyte. A highly sensitive test correctly detects a large proportion of true positive cases, minimizing the chances of false negative results. Thus, sensitivity is crucial for diagnosing infectious diseases.

We evaluated the performance of colloidal gold and Estapor® Red Intense microspheres as detector particles in a FluA antigen LFIA. Colloidal gold nanoparticles were conjugated by passive adsorption, and Estapor® Red Intense microspheres were conjugated using the standard two-step EDC/Sulfo-NHS covalent coupling process (see Experimental Section for detailed procedure). We compared the performance of both detector particles in terms of sensitivity, precision, speed of detection and raw material cost.

Sensitivity of Detection

The sensitivity of both detector particles was assessed by adding increasing concentrations of target analyte to the test. The results are shown for both qualitative visual detection (**Figure 2**) and quantitative detection using a test strip reader (**Figure 3**).

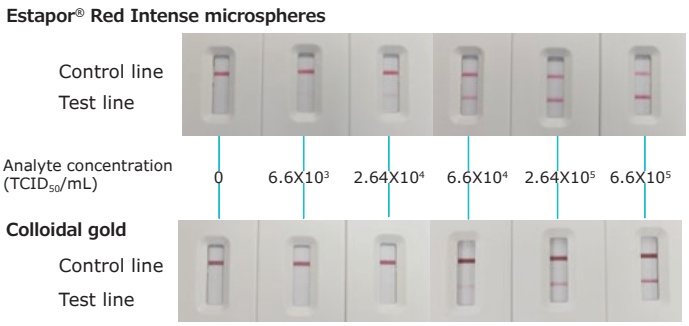

Figure 2 – Visual detection of increasing FluA analyte concentration with Estapor® Red Intense microspheres or colloidal gold.

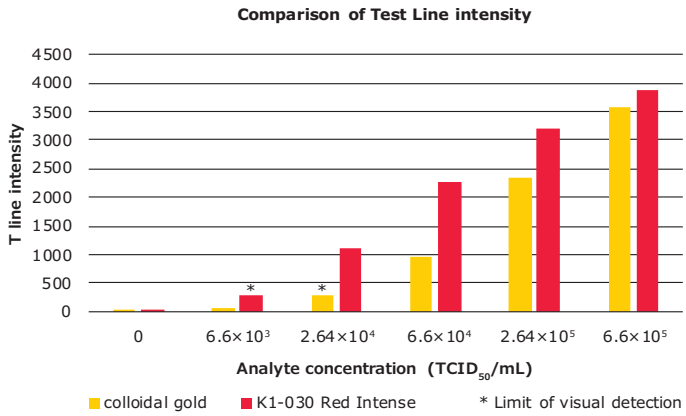

Figure 3 – Quantitative determination of test line intensity for increasing FluA analyte concentration using Estapor® Red Intense microspheres (red bars) and colloidal gold (yellow bars).

For the blank sample (0 $TCID_{50}$/mL), both colloidal gold and Estapor® Red Intense microspheres produced negative results, showing no sign of false positive signals. The limit of visual detection using the Red Intense microspheres was as low as $6.6x10^3$ $TCID_{50}$/mL, while colloidal gold was not visible at this concentration at 15 minutes (see Speed of Detection). The lowest concentration visually detectable with colloidal gold was $2.64x10^4$ $TCID_{50}$/mL. At higher concentrations, both detector particles gave visible signals, but Estapor® Red Intense microspheres displayed superior color intensity.

These visual results were confirmed by quantification of test line intensity using a LFIA strip reader. Regardless of the analyte concentration, Estapor® Red Intense microspheres gave higher test line intensity values. For the weak positive samples ($6.6x10^3$ $TCID_{50}$/mL and $2.64x10^4$ $TCID_{50}$/mL), the test line intensity with the Estapor® Red Intense microspheres was 3.7 to 4 times greater than observed with colloidal gold. For higher concentrations, the intensity of the test line was too high and saturated the reader's detector.

Overall, the sensitivity achieved with the Estapor® Red Intense microspheres was much higher than achieved with colloidal gold, allowing for the detection of lower analyte concentration and unambiguous visual interpretation of the results.

Precision of Detection

While having a low limit of detection is a key element, it is also crucial that a LFIA produces reliable and reproducible results. Thus, we assessed the precision of our FluA LFIA by repeating the test ten times for medium and strong positive results. Once again, results were analyzed both qualitatively (**Figure 4**) and quantitatively (**Table 2**).

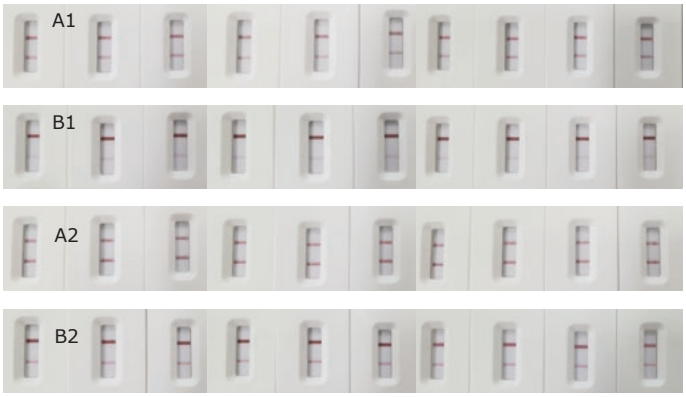

Figure 4 – Precision of visual detection for ten repetitions.

A1 – Estapor® Red Intense microspheres, analyte concentration = $6.6x10^4$ $TCID_{50}$/mL
B1 – colloidal gold, analyte concentration = $6.6x10^4$ $TCID_{50}$/mL
A2 – Estapor® Red Intense microspheres, analyte concentration = $2.64x10^5$ $TCID_{50}$/mL
B2 – colloidal gold, analyte concentration = $2.64x10^5$ $TCID_{50}$/mL

Table 2 - Comparison of test line precision between colloidal gold and Estapor® Red Intense microspheres

	Colloidal Gold		Estapor® Red Intense Microspheres	
Analyte Concentration ($TCID_{50}$/mL)	**$6.6X10^4$**	**$2.64x10^5$**	**$6.6x10^4$**	**$2.64x10^5$**
Average test line intensity	1075	2440	2414	3655
Std Dev	84	126	186	201
CV	7.8 %	5.2 %	7.7 %	5.5 %
Average CV	6.5 %		6.6 %	

As shown, Estapor® Red Intense microspheres and colloidal gold were comparable in terms of precision, with average coefficients of variation of 6.6 % and 6.5 %, respectively, and no clear visual perception difference.

Speed of Detection

The speed of test line appearance is an important factor to consider when selecting the raw materials for a LFIA. It can provide insights into the performance and efficiency of the test. Comparing the speed of test line appearance aids in selecting the most appropriate LFIA design features for accurate and timely results.

In the FluA assay model, the speed of appearance was assessed by measuring the time needed to visually detect the test line with the naked eye. **Figure 5** illustrates the test line appearance time for both Estapor® Red Intense microspheres and colloidal gold. The test line appeared more quickly with Estapor® Red Intense microspheres, allowing for faster reading of the test results. This was particularly true for low analyte concentrations where Estapor® Red Intense microspheres gave results more than five times faster than colloidal gold.

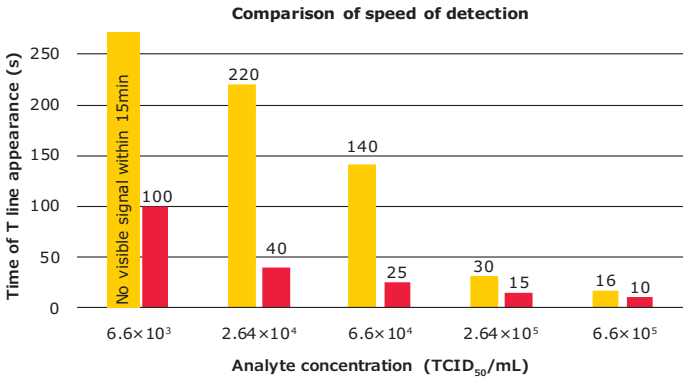

Figure 5 – Comparison of time to visual test line appearance for Estapor® Red Intense microspheres (red bars) and colloidal gold (yellow bars) for different analyte concentrations.

Cost Comparison

Comparing the cost of each detector particle is not an easy task as it has many different parameters like batch size, volume purchased, targeted analyte, internal raw material production, coupling procedure, etc. **Table 3** provides the general cost of the major raw materials used in the conjugation process. Estapor® Red Intense microspheres give a 24 % cheaper conjugated detector particle in terms of raw materials.

Table 3 - Cost Comparison of Raw Materials

Material	Price	Cost in 1 test (colloidal gold)	Cost in 1 test (Red Intense microspheres)
Supplier A colloidal gold	286 €/100mL	0.087 c€	-
Estapor® Red Intense microspheres	400 €/g	-	0.003 c€
Merck EDC	27 €/g	-	0.04 c€
Merck Sulfo-NHS	301 €/g	-	0.42 c€
Fapon FluA antibody (detector)	64 €/mg	1.56 c€	0.79 c€
Total Conjugate Raw Material cost per test		1.65 c€/test	1.25 c€/test

Conclusion

In conclusion, this report compares the performance of Estapor® K1-030 Red Intense microspheres with colloidal gold as detector particles in a model FluA LFIA.

The sensitivity of the Estapor® Red Intense microspheres was found to be superior to colloidal gold in detecting lower analyte concentrations and displaying stronger test line intensity. The precision of both particles was comparable, showing reliable and reproducible results.

Estapor® Red Intense microspheres also produced faster appearance of the test line, allowing for quicker reading of the results, particularly at low analyte concentrations.

Overall, the findings support the conclusion that Estapor® K1-030 Red Intense microspheres enhance the performance and reliability of LFIA.

Experimental Section

Materials and Reagents

Material Information List			
No.	**Product name**	**Supplier**	**Cat. No.**
1	K1-030 Red Int COOH PS 5 %	Merck	**FR180380637-1**
2	Gold Colloid	Supplier A	**N/A**
3	Hi-Flow™ Plus HF90	Merck	**SHF0900225**
4	EDC	Merck	**E7750**
5	Sulfo-NHS	Merck	**56485**
6	FluA antibody (detector)	Fapon	**FLUA-REAB-G1-006**
7	FluA antibody (capture)	Fapon	**FLUA-REAB-G1-009**
8	FluA QC Positive Control	Artron	**C77-P1**
9	Goat Anti-(Mouse IgG)	Fapon	**N/A**
10	Bovine Serum Albumin	Merck	**V900933**
11	Glass fiber (conjugate pads)	Merck	**GFDX103000**
12	Polyester fiber (sample pads)	Ahlstrom	**6613**
13	Absorbent pads	Shanghai Goldbio	**CH37**
14	Backing cards	Hangzhou Ruijian	**RY-PVC-6cm**
15	Cartridges	Nantong Manzhen	**KB07**

Equipment

Material Information List			
No.	**Product name**	**Supplier**	**Cat. No.**
1	Lateral Flow Reader	Jensen	**GIC-S2011-C25**
2	Ultrasonic Homogenizer Sonicator	Ningbo Scientz	**JY92-IIN**
3	Centrifuge	Thermo	**Sorvall Legend Micro 21R**

Test Protocol

Striping membrane with capture and control antibodies

1. Control Line - Goat anti-(mouse IgG) is diluted to 1 mg/mL using 10 mM PBS, 2 % trehalose (pH 7.4).
2. Test Line - Influenza A antibody is diluted to 1 mg/mL in 10 mM PBS, 2 % trehalose (pH 7.4).
3. Remove the release liner covering the membrane track; the other release liners remain on the card.
4. Place a 30-cm long membrane strip onto the card.
5. Stripe the test line and control line solutions onto the membrane at 1 µL/cm.
6. Dry striped membrane at 37 °C in a drying oven overnight.
7. Store membrane samples in a sealed foil bag with desiccant until needed.

Anti-FluA antibody conjugation to Estapor® Red Intense microspheres and conjugate pad preparation

1. Add 100 µL of Estapor® Red Intense microspheres (5 %) to a 2-mL low protein adsorption centrifuge tube.
2. Add 400 µL 50 mM MES (pH 6.0) to the tube and mix well.
3. Centrifuge at 14,000 rpm for 20 minutes.
4. Remove the supernatant and disperse the microspheres in 0.5 mL 50 mM MES (pH 6.0).
5. Sonicate the microspheres for 60 seconds to ensure that they are not aggregated.
6. Prepare 20 mg/mL EDC and 20 mg/mL Sulfo-NHS in 50 mM MES (pH 6.0).
7. Rapidly add 17.5 µL of 20 mg/mL EDC and 17.5 µL of 20 mg/mL Sulfo-NHS to the microsphere suspension from Step 5.
8. Mix and incubate on a rotator for 20 minutes.
9. Centrifuge at 14,000 rpm for 20 minutes.
10. Remove the supernatant and disperse the microspheres in 0.5 mL 50 mM MES (pH 6.0).
11. Sonicate the microspheres for 60 seconds to ensure that they are not aggregated.
12. Add 0.4 mg of detector antibody; mix and incubate on a rotator for 2 hours at room temperature.
13. Centrifuge at 14,000 rpm for 20 minutes.
14. Remove the supernatant and disperse the microspheres in 0.5 mL blocking buffer (50 mM Tris, pH 8.0, 0.5 % casein).
15. Sonicate the microspheres for 60 seconds; mix and incubate on a rotator for 2 hours.
16. Centrifuge at 14,000 rpm for 20 minutes.
17. Remove the supernatant, and disperse the microspheres in 0.5 mL storage buffer (50 mM Tris, pH 8.0, 3 % BSA, 10 % trehalose) by sonication. The microsphere concentration is 1 % w/v. Store at 2-8 °C for later use.
18. Prepare conjugate diluent buffer (50 mM Tris, 0.5 % casein, 0.5 % polyvinylpyrrolidone 10, 10 % sucrose, 2.5 % trehalose, pH 8.0).

19. Dilute 25 µL of the 1 % w/v conjugated microspheres (from Step 17) with 225 µL of conjugate diluent buffer (from Step 18) to obtain 0.25 mL of conjugated microspheres at a concentration of 0.1 % w/v.
20. Spray the 0.1 % w/v conjugate solution onto the blank glass fiber pad (1 cm x 30 cm) at a rate of 3 µL/cm of pad length.
21. Dry the conjugate pad at 37 °C overnight in the drying oven. Store the dried pad in a sealed foil bag with desiccant.

Note: The amount of antibody added in Step 12 and the dilution ratio of microspheres in Step 19 can be adjusted to meet performance and cost requirements.

Anti-FluA antibody conjugation to colloidal gold and conjugate pad preparation

1. Add 5 mL of 40-nm colloidal gold stock solution to the tube.
2. Adjust pH to 8.0 ± 0.1 with 0.1 M K_2CO_3.
3. Add 40 µg antibody to the tube and stir at room temperature for 20 minutes.
4. Add 50 µL of blocking buffer (10 % BSA in Milli-Q® water, pH 6.8).
5. Stir at room temperature for 20 minutes.
6. Centrifuge the conjugate at 6000 rpm for 20 minutes.
7. Remove the supernatant, and resuspend in 0.1 mL of Storage Buffer (20 mM Tris, 1 % BSA, pH 8.2)
8. Vortex to fully resuspend the conjugate.
9. Store the conjugate at 2-8 °C until use.
10. Prepare conjugate diluent buffer (50 mM Tris, 0.5 % casein, 0.2 % polyvinylpyrrolidone 10, 0.3 % Tetronic® 1307, 20 % trehalose, pH 8.0).
11. Prepare 1 mL of conjugated microspheres at a concentration of OD 5.

Preparation of conjugated microspheres solution				
Final Concentration	Dilution Factor	Conjugated microspheres	Volume of conjugate diluent buffer	Final Volume
OD 5	8	125 µL	875 µL	1000 µL

12. Pipet 900 µL of conjugate solution onto the polyester fiber pad (1 cm x 30 cm, pretreated with 50 mM Tris, 0.5 % casein, 1 % polyvinylpyrrolidone 10, 0.05 % Tween® 20, pH 8.2) using a micropipette.
13. Dry the conjugate pad at 37 °C overnight in a drying oven.
14. Store the pad in a sealed foil bag with desiccant.

Sample pad preparation

1. Prepare sample pad solution: 10 mM Tris, 1 % Tween® 20, pH 8.2.
2. Soak one glass fiber pad with sample pad solution (~50 mL), making sure that there are no entrapped air bubbles.
3. Place the treated pad on a wire tray to dry overnight at 37 °C in a drying oven.
4. Cut off edges and cut into strips 1.7 cm wide for storage.
5. Store the sample pad in a sealed foil bag with desiccant.

Absorbent pad preparation

Cut absorbent pad into strips 1.7 cm wide.

Cards Assembly

1. Place the conjugate pad just below the membrane with 2 mm overlap onto the membrane. Press down on the conjugate pad along the length of the card to ensure good contact with the adhesive.
2. Place the sample pad onto the exposed adhesive so that one edge aligns with the edge of the adhesive card. The other edge of the sample pad should overlap onto the conjugate pad by approximately 4 mm. Press down on the sample pad along the length of the card to ensure good contact with the adhesive.
3. Place the absorbent pad above the membrane with 2 mm overlap on the upper edge of the membrane.
4. Cut the sample cards into 4 mm wide test strips (**Figure 6A**), then install the strips in cartridges (**Figure 6B**).

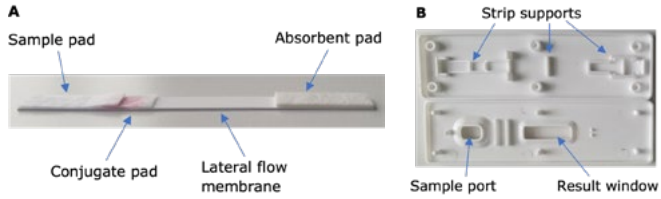

Figure 6 – A: Lateral flow strip assembly; B: Internal construction of cartridge

Analyte solution preparation

1. Prepare sample dilution buffer: 50 mM Tris, 3.5 % NaCl, 1 % BSA, 0.1 % Triton™ X-100, pH 8.5. This buffer is used to dilute the FluA positive control.
2. The FluA H1N1 positive control is supplied at a concentration of 6.6×10^6 $TCID_{50}$/mL (refer to the Certificate of Analysis). This is diluted to a concentration of 6.6×10^5 $TCID_{50}$/mL.
3. Use the 6.6×10^5 $TCID_{50}$/mL stock solution to prepare 5 mL of analyte solutions at concentrations of 2.64×10^5 $TCID_{50}$/mL, 6.6×10^4 $TCID_{50}$/mL, 2.64×10^4 $TCID_{50}$/mL, and 6.6×10^3 $TCID_{50}$/mL

Preparation of conjugated microspheres solution				
Final Concentration	**Dilution Factor**	**Volume of positive control**	**Volume of sample dilution buffer**	**Final Volume**
6.6×10^5 $TCID_{50}$/mL	10	500 µL	4500 µL	5000 µL

Preparation of 2.64×10^5 $TCID_{50}$/mL, 6.6×10^4 $TCID_{50}$/mL, 2.64×10^4 $TCID_{50}$/mL, 6.6×10^3 $TCID_{50}$/mL Dilutions				
Final Concentration	**Dilution Factor**	**Volume of 6.6×10^5 $TCID_{50}$/mL solution (prepared above)**	**Volume of conjugate diluent buffer**	**Final Volume**
2.64×10^5 $TCID_{50}$/mL	2.5	2000 µL	3000 µL	5000 µL
6.6×10^4 $TCID_{50}$/mL	10	500 µL	4500 µL	5000 µL
2.64×10^4 $TCID_{50}$/mL	25	200 µL	4800 µL	5000 µL
6.6×10^3 $TCID_{50}$/mL	100	50 µL	4950 µL	5000 µL

Running FluA assay and interpreting the results

1. Add 80 µL of analyte solution to the base of the sample pad and start the timer.
2. After 15 min, read the signal by visual inspection (see table below for visual interpretation).
3. Insert the test into the lateral flow reader and record the signal values.

Visual aspect			
Interpretation	Negative	Positive	Invalid
Comments	Presence of only one colored band (control line “C”).	Presence of two, colored lines (test line “T” and control line “C”), even if the test line is faint.	Control line “C” is not visible, even if the test line is present

References

1. https://www.sigmaaldrich.com/FR/fr/product/mm/fr180380637
2. https://www.sigmaaldrich.com/FR/fr/product/mm/fr180380638
3. https://www.sigmaaldrich.com/FR/en/technical-documents/technical-article/clinical-testing-and-diagnostics-manufacturing/ivd-manufacturing/estapor-red-intense-microspheres-comparison
4. https://www.selectscience.net/editorial-articles/from-gold-to-bold-new-intense-color-microspheres-for-lateral-flow-assays/?artID=59868
5. https://www.sigmaaldrich.com/FR/en/services/ivd-development-manufacturing/lateral-flow-services
6. Lateral flow test engineering and lessons learned from COVID-19|Nature Reviews Bioengineering

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