Application Note

Immuno Diagnostics

Effect of Magnetic Bead Redispersion on Automated Chemiluminescent Assays

Authors

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Abstract

Homogeneous magnetic bead redispersal and solids content are two intrinsically linked parameters that are easily overlooked in automated chemiluminescent immunoassay (CLIA) applications due to time constraints and elevated costs of the material. Bead losses during protein conjugation, incomplete ligand coupling due to heterogenous bead dispersion, or inaccurate addition of bead solids will contribute to irreproducible, misleading data that could result in detrimental interpretation of the assay outcome. This application note demonstrates how considering magnetic bead redispersion can improve the consistency of assay data, and highlights the impact of poor magnetic bead solids calculations in CLIA applications.

Introduction

Superparamagnetic microparticles, or "magnetic beads," are commonly used as separation supports for analysis of complex heterogeneous biological matrices, including blood, urine, food, or tissue. Magnetic beads are often functionalized with biomolecules to make them selective for a specific target. The incorporation of magnetic beads as solid phases in CLIA can present many challenges to assay developers. Optimization of several parameters is often necessary to achieve assay reproducibility, maximize signal-to-noise ratio, and avoid false positives or negatives. Suboptimal assay data can be the result of complex protein interactions between the antibody pair selected for sandwich assays. However, CLIA performance can also be affected significantly by poor bead-handling techniques.

The purpose of this application note is to demonstrate that successful redispersion of Agilent LodeStars magnetic beads leads to minimal variation in sample concentration determined by a subsequent CLIA assay (coefficient of variation [CV] < 3%). Gravimetric analysis was performed to determine redispersion efficacy where variation in solids content of more than 3% was recorded. When these variations were incorporated into an automated CLIA assay, impactful results were observed favoring the likelihood of false positives or negatives. Optical microscopic imaging was also used to illustrate the difference between homogenous and heterogeneous bead dispersion.

Sample preparation

LodeStars 2.7 Streptavidin beads of known solids content were fully redispersed with a bottle roller for more than 16 hours at room temperature at 60 revolutions-per-minute (rpm). Multiple 100 mL volumes of this fully redispersed material were aliquoted into 125 mL sample containers and stored at 2 to 8 °C for 3 months. This storage period allowed the beads to sediment. Use of sedimented beads permits better assessment of the effect on solids measurements if dispersion is not handled correctly. The sample containers prepared in this manner were further treated as described in the following section to explore selected process variables.

Results and discussion

Redispersion of LodeStars 2.7 Streptavidin beads can be carried out using numerous methods (e.g., rolling, shaking by hand). However, homogeneous redispersion is not achieved by all methods. Figure 1 shows the effect of the aliquoting process if the beads have not been fully redispersed. Poor dispersion can cause differing concentrations of beads throughout a sample, resulting in varying solids content, depending on the location sampled.

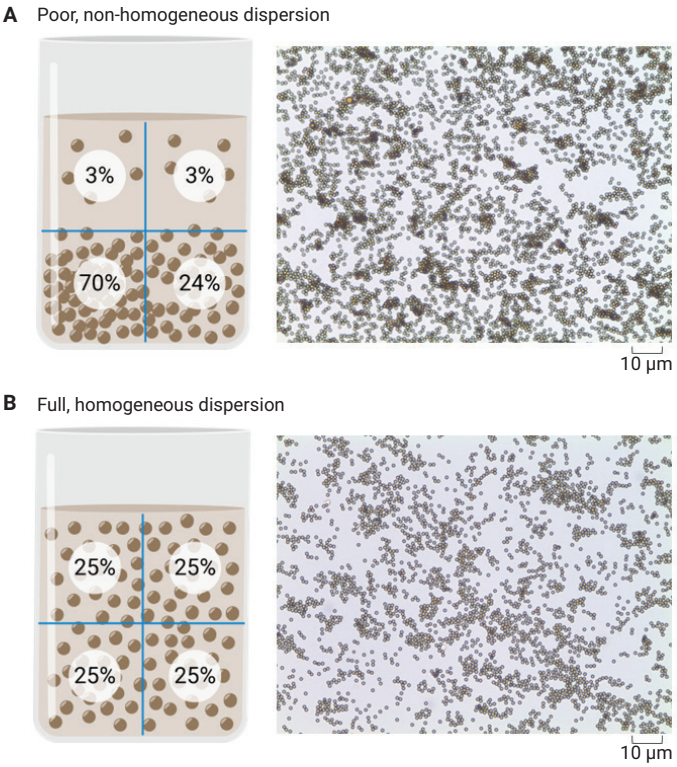

Figure 1. Optical microscope images of samples that are (A) poorly redispersed and (B) fully redispersed.

Dispersion methods

A range of common variables related to dispersion were explored to demonstrate the importance of controlling them or implementing strategies to mitigate them:

- Sampling location and order
- Redispersion time
- Time in suspension
- Mixing speed (rpm)
- Container fill level
- Bottle volume

Sampling location and order: Three sampling heights were selected for this study (Figure 2):

- Top (T)
- Middle (M)
- Bottom (B)

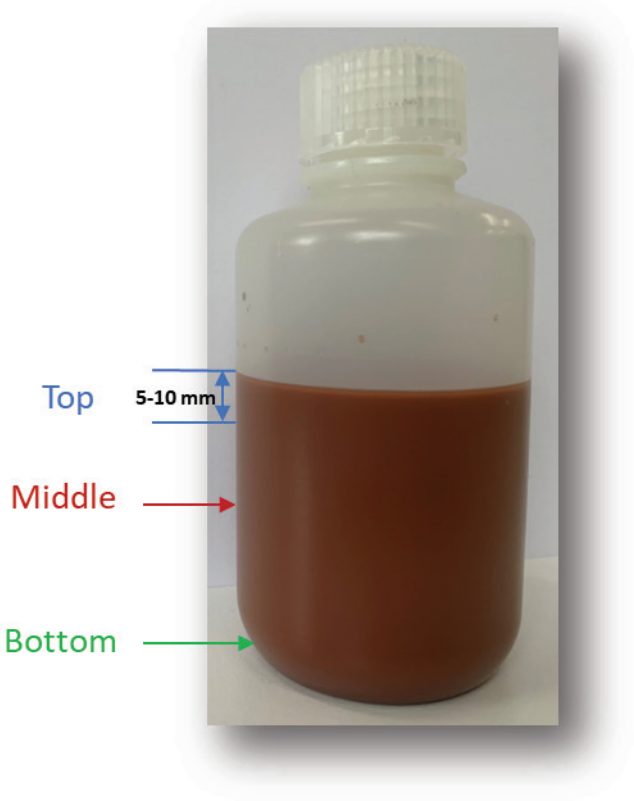

Figure 2. Sampling locations.

Beads with known concentrations were dispersed at room temperature for one hour and overnight (> 16 hours), and samples were taken from the three locations (Figure 2). The order of sample aliquoting was also reversed to assess the effect of this factor. Measurements were made in duplicate and variation acceptance was set to ± 3%.

Figure 3 shows that sampling order has less effect than sampling location. Sampling from the bottom of the container gave inaccurate results, and therefore the remaining experiments were carried out by aliquoting from the top and middle locations only.

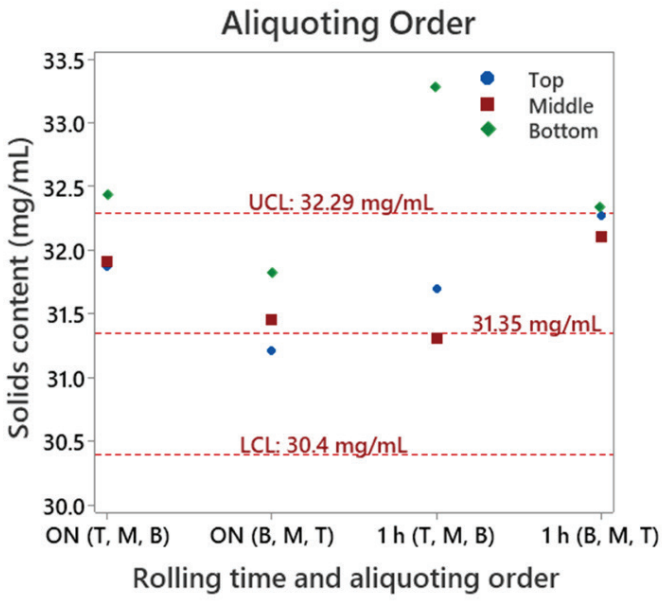

Figure 3. Effect of sampling location and aliquoting order on solids content (ON = overnight, UCL = upper control limit, LCL = lower control limit).

Redispersion time: LodeStars 2.7 Streptavidin beads of a known concentration were treated at room temperature with different dispersion times and techniques to evaluate the effect of these variables on solids content (Figure 4). When the beads were rolled overnight, tight results were obtained. Slight differences were observed when the beads were rolled for one hour, but results were within the target 3% variation. However, when beads were agitated by hand, inaccurate concentrations were registered. This result demonstrates that beads must be rolled for at least one hour to maintain accurate values for solids content.

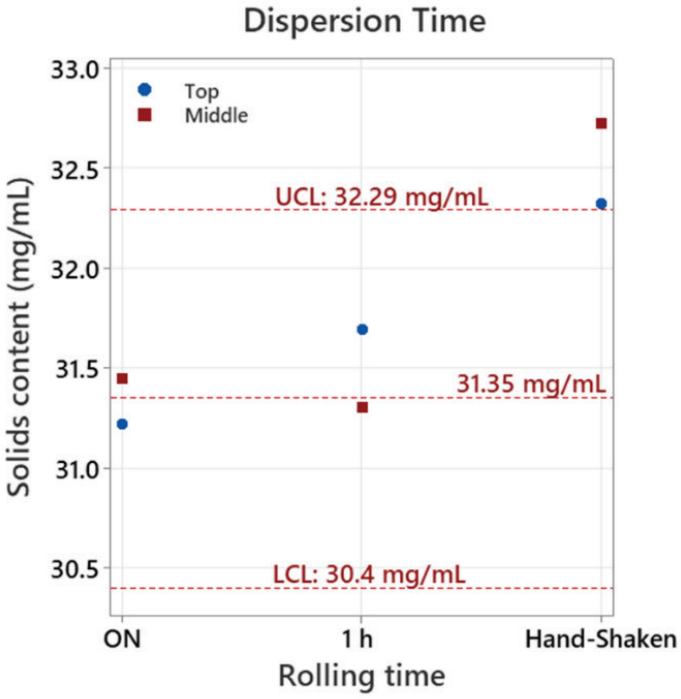

Figure 4. Effect of dispersion time on bead redispersion.

Time in suspension: Two bottles of LodeStars 2.7 Streptavidin beads were rolled at room temperature for over one hour and overnight. The beads were removed from the bottle roller and sampled at timed intervals to estimate the suspension time of the beads without altering their concentration.

Figure 5A shows that overnight redispersion keeps the beads in suspension longer, with minimal variation in concentration. Overnight rolling improves the colloidal stability of the beads, decreasing the sedimentation rate so that accurate solids values are still obtained after 10 minutes with the bottle in a standing position.

When time was a constraining factor, accurate solids were measured after rolling the beads for one hour followed by immediate sampling (Figure 5B).

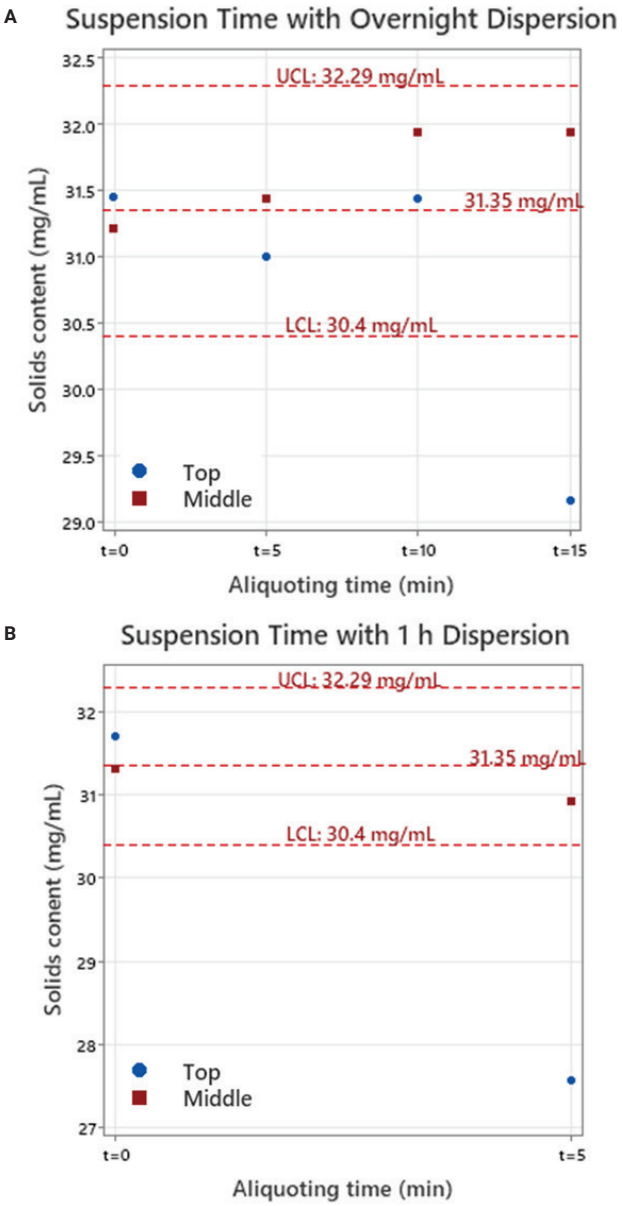

Figure 5. Effect of suspension time on solids content after redispersing beads (A) overnight or (B) for one hour.

Mixing speed: The speed of the bottle roller was investigated to achieve a successful redispersion of the beads. Different bottles of known concentration were rolled over one hour and overnight at three speeds and room temperature.

The obtained results show that roller speed has little effect on bead redispersion if the beads are rolled overnight (Figure 6A). However, when the beads are rolled for one hour, the roller speed should not be greater than 30 rpm, as the time is not enough to achieve full redispersion (Figure 6B).

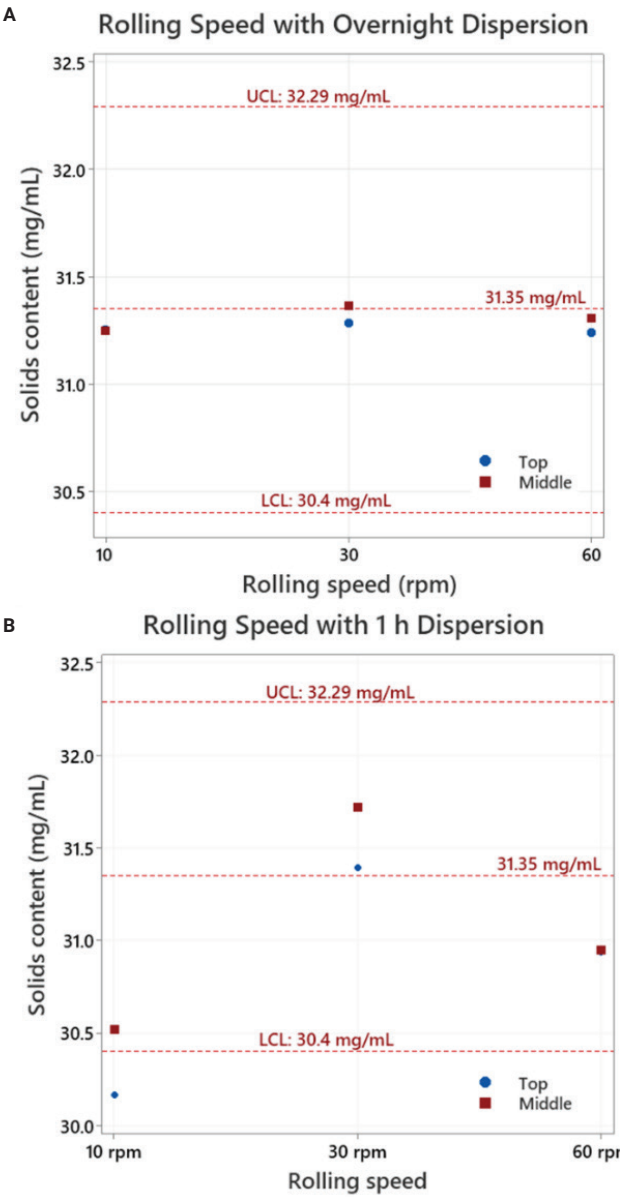

Figure 6. Effect of bottle roller speed on solids content after redispersing beads (A) overnight or (B) for one hour.

Container fill level: LodeStars 2.7 Streptavidin beads are supplied with a gap of approximately 20% between the slurry and the lid of the bottle to allow sufficient movement and achieve optimal dispersion. This gap is also known as headspace.

To assess the effect of different fill levels on bead redispersion, three headspaces over two time periods were studied.

The selected headspaces were:

- < 5%: Over-filled bottle
- > 20%: Commercial product conditions
- > 90%: Last milliliter of beads

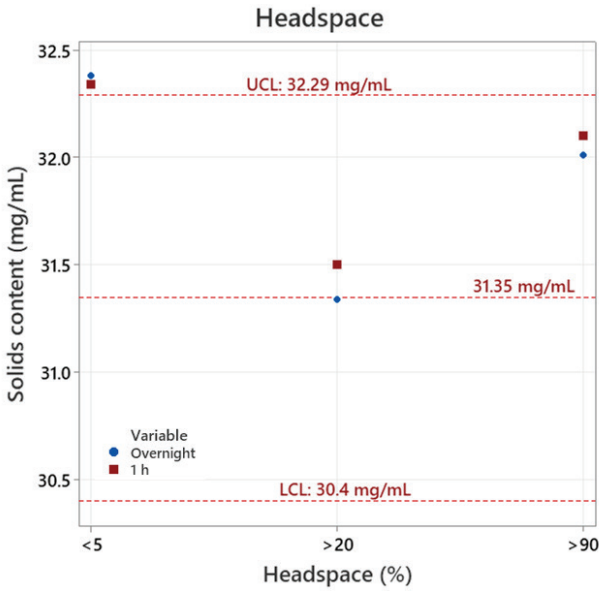

Figure 7. Effect of headspace on solids content.

Figure 7 shows that both one hour and overnight rolling yielded inaccurate results at the high fill levels, demonstrating the importance of headspace in achieving homogenous dispersion. On the other hand, small quantities can be adequately redispersed in large containers with both rolling times.

Bottle volume: Different bottle sizes with headspaces greater than 20% were rolled overnight and over one hour at room temperature. Figure 8 shows that the volume of the bottle does not affect bead redispersion, as solids content values with CV < 3% were achieved in all cases.

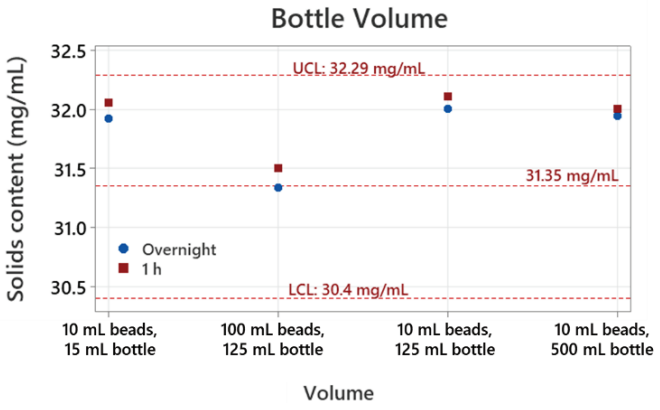

Figure 8. Effect of bottle volume on solids content.

CLIA assay

An automated CLIA platform running an intact parathyroid hormone (i-PTH) assay (IDS Systems) was used to demonstrate the effect of inaccurate bead solids addition.

Beads rolled at room temperature overnight at 60 rpm were chosen as the reference signal. Results for different situations and redispersion methods are shown in Figure 9. As has been demonstrated in this study, when full redispersion is not achieved, solids content can vary by ± 3.5% of the original concentration. Although this variance is not large, when it is present in a CLIA application, the assay results can vary more than 10% from the real signal. This discrepancy can lead to inaccurate assay results and affect individual outcomes.

Use of redispersion times less than one hour or use of a redispersion method other than a bottle roller (e.g. vortex mixer, hand shaken) can lower the coefficient of variation below 10%. However, these changes would risk the obtention of false positives or negatives.

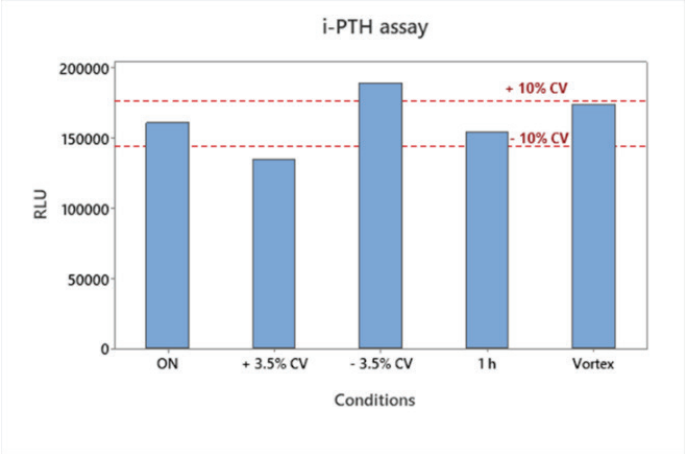

Figure 9. i-PTH assay performance with nonhomogeneously redispersed beads.

Conclusion

This application note illustrates the importance of optimizing magnetic bead redispersion techniques and associated bead solids calculations to achieve higher reproducibility and higher confidence in resulting chemiluminescent immunoassay (CLIA) applications.

For optimal use of Agilent LodeStars beads, Agilent recommends overnight redispersion (> 16 hours) to mitigate factors such as aliquoting times and rolling speed that can compromise the bead concentration and ultimately affect the performance of CLIA assays.

Although LodeStars 2.7 Streptavidin beads were selected for this application note, these guidelines should be applied to other Agilent magnetic products (LodeStars Original and LodeStars High Bind carboxyl and streptavidin beads) for optimal assay performance.

Ordering information

Table 1. Ordering information.

Description	LodeStars Original Beads (10 mg/mL)		LodeStars High Bind Beads (50 mg/mL)	
Streptavidin Beads	2 mL	PL6727-1001	2 mL	PL6827-1001
	10 mL	PL6727-1003	10 mL	PL6827-1003
	100 mL	PL6727-1005	100 mL	PL6827-1005
	–	–	400 mL	PL6827-1006
	800 mL	PL6727-1007	–	–
Description	**LodeStars Beads (30 mg/mL)**		**LodeStars High Bind Beads (50 mg/mL)**	
Carboxyl Beads	2 mL	PL6727-0001	2 mL	PL6827-0001
	10 mL	PL6727-0003	10 mL	PL6827-0003
	100 mL	PL6727-0005	100 mL	PL6827-0005
	400 mL	PL6727-0006	400 mL	PL6827-0006
	800 mL	PL6727-0007	–	–

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