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The highs and lows of turbidimetry: assessing its suitability for Point of Care immunoassays

By Nick Collier, Chief Technology Officer

Turbidimetry is an attractive option for point of care immunoassay diagnostic devices because of its simplicity, high-sensitivity and fast turn-around times. However, as the breadth of diagnostics available at Point of Care (PoC) increases and the demand that they should emulate the sensitivity and accuracy of central lab results remains, so immuno-turbidimetry may reach its limits.

In this article, Nick Collier, CTO Sagentia-Medical, assesses those limits and suggests how they might be addressed.

¬ Assessing the value of turbidimetry

Particle Enhanced (PE) agglutination is a frequently used technique for the detection of analytes. It provides very high sensitivity in a simple 'one pot' format thus removing the need for complex automation. The high surface area of the particles dispersed throughout the sample also results in a quick time to result.

Typically antibodies are bound to the surface of latex particles and form aggregates when an antigen is present which has multiple binding sites. The sample and reagents are combined and the degree of particle agglutination is observed optically. This can be a kinetic assay where the rate of agglutination is measured or an end-point assay where the equilibrium agglutination is measured on completion.

The particle agglutination can be determined either by turbidimetry or nephelometry. Turbidimetry is the more straightforward method and measures the attenuation in light transmitted through the sample.

¬ PE assays in practice

PE turbidimetry can be found both in high-throughput central laboratory instruments and in PoC tests. At PoC, the simple one-pot format of immunoturbidimetry is very attractive due to its simplicity and the low-cost

of the hardware. An example assay workflow (in an end-point assay) is as follows:

Add whole blood sample to a tube of lysis buffer

Mix and wait for complete lysis of red blood cells

Take a blank reference measurement of optical attenuation

Add the coated latex particles and mix

Wait for the reaction to go to completion

Take an end-point optical measurement of optical attenuation

Use a previously established calibration to convert the attenuation to analyte concentration

So on the face of it turbidimetry proves a simple and practical solution for point of care requirements, but potentially it isn't ideally suited for the detection of all analytes.

Immunoturbidimetry sweetspots and limitations

In recent years the menu of available PoC tests has been increasing and these tests are required to provide actionable diagnostic results comparable to those from a central lab. There is a desire to achieve both a low limit of detection as well as a large dynamic range that covers the full clinical range. For analytes where the required limit of detection is high (>uM) and the dynamic range is small (in the order of 100 to 300), immunoturbidimetry works well.

There are some analytes, however, which don't fall into these parameters. Procalcitonin (PCT), for example, which is an indicator of systemic bacterial infection, shows a wide clinical range[1]:

- Normal levels of PCT <0.15ng/mL
- Levels between 0.15 and 2.0 ng/mL do not exclude infection – they might suggest a non-systemic infection
- Levels above >2.0ng/mL are highly suggestive of systemic bacterial infection
- Levels can be >>150 ng/mL

The following assays all seek to detect PCT and therefore have to handle wide measurement ranges but use chemiluminescence to achieve the analyte detection:

- The Siemens chemiluminescent assay, ADVIA centaur BRAHMS PCT, has a measuring range of 0.02ng/mL: to 75ng/mL. This is a dynamic range of 3750 with a minimum level of detection of 1.5pM.
- Similarly, the Abbott chemiluminescent assay, Architect BRAHMS PCT, has a measuring range of 0.02ng/mL: to 100ng/mL (without dilution). This is a dynamic range of 5000 with a minimum level of detection of 1.5pM[2].

There are, however, several providers of reagents for PCT assays to be run on central laboratory instruments that do use latex enhanced immunoturbidimetry:

- PCT Liquicolor from EKF diagnostics - 0.17-50ng/mL with a dynamic range of 2943[3]
- Diazyme PCT LOD=0.16ng/mL LOQ=0.2ng/mL upper reported linear range 52ng/mL, a dynamic range of 260 with a LOD of 2pM[4].

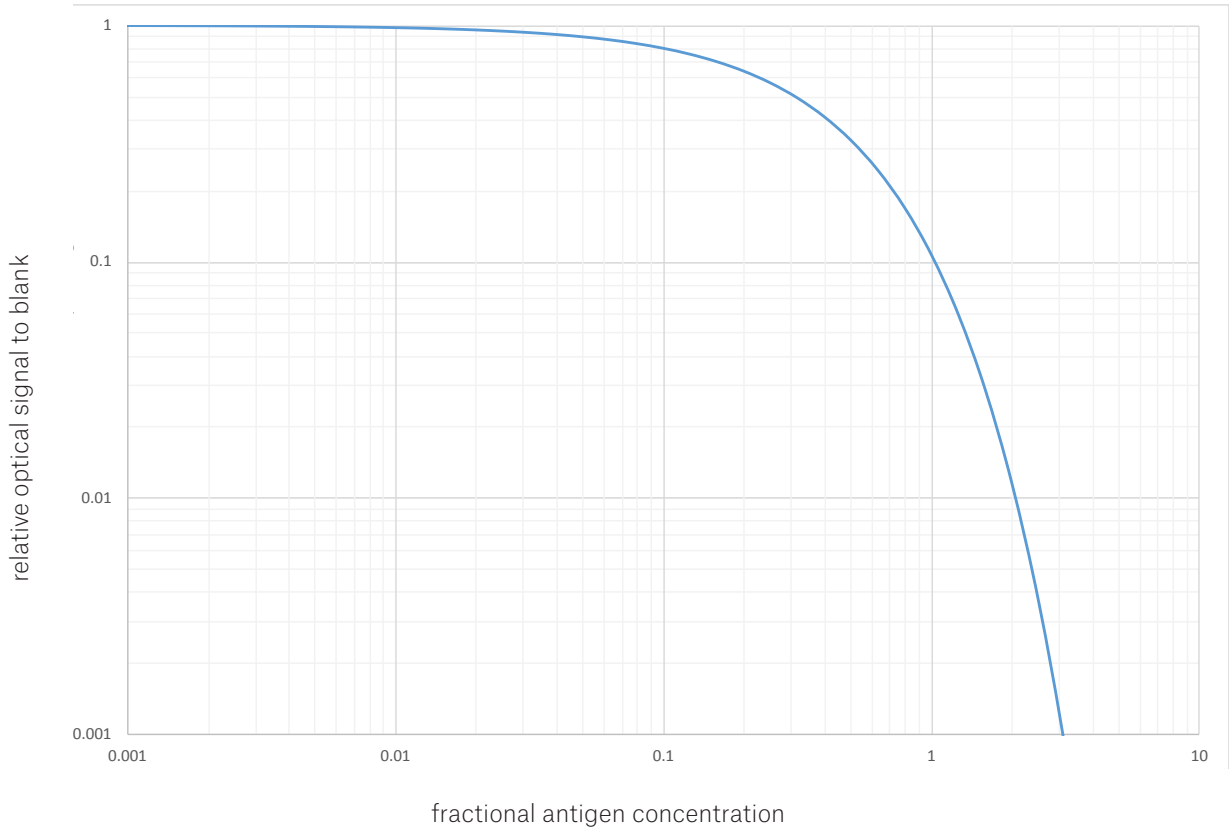

Figure 1 turbidimetry: the limits of dynamic range in practice

The dynamic ranges of these assays using immunoturbidimetry are significantly less than the assays using chemiluminescence and obviously less than the clinical range of PCT. This suggests that the level of detail they will give to the clinician will be limited.

So we see that immunoturbidimetry on the one hand is suited to PoC settings as it is a simple one-pot assay. On the other hand, it has insufficient dynamic range and sensitivity (outside that range) to compete with the standard central laboratory PCT tests that use other methods/technologies such as chemiluminescence.

So what limits the performance of immunoturbidimetry and what are the alternatives?

¬ Investigating dynamic range

Immunoturbidimetry is based upon the use of latex particles with a size less than the wavelength of light. When these particles agglutinate the amount of light scattered increases significantly due to the increase in particle size. We can detect this as either a reduction in forward transmitted light or an increase in light scattered to the sides. It allows a relatively simple optical setup, however it is non-linear and this limits the dynamic range that can be achieved in practice.

In the graph above a maximum antigen concentration has been chosen that avoids the hook effect and which provides a 2% optical signal, i.e the forward beam is mostly attenuated. We see two behaviours, at low antigen concentration very little light is scattered and it is necessary to detect a small change on a large background. At high concentrations the signal is rapidly attenuated and the slope of the line becomes very steep. The net effect is that the dynamic range is limited by noise and experimental drift.

The loss of sensitivity at high concentrations can be compensated somewhat by using measurements taken at earlier reaction times before the assay has gone to completion. However this also introduces variability due to reagents, temperature and timing accuracy.

In practice this means that in a PoC setting, except for a limited range, the test is effectively binary. Normal

patients' results will manifest as "below measurable range" and patients with a high level will simply get "high" as a result – with the risk that the hook effect could come into play delivering a false negative.

There are a number of possibilities for increasing the dynamic range whilst keeping the 'one pot' approach

¬ Options for increasing the dynamic range

There are a number of possibilities for increasing the dynamic range whilst keeping the 'one pot' approach:

1. **Multiple sample diultions**
   Admittedly this is not really a one-pot solution, but this is the standard approach to increasing dynamic range, at least on the upper end. It allows us to measure high concentrations and optimize the setup for low concentrations. However, it does not necessarily improve the lower limit of detection. It also adds complexity and requires additional reagents, plasticware and sample undermining the suitability of immunoturbidimetry for the PoC setting.

2. **Multiple wavelengths**
   The degree of light scattering depends on wavelength, we can therefore use two or more wavelengths with one optimized for low concentrations and the other for high concentrations and use one of two calibration curves based upon the determined concentration range. This gives a modest improvement in dynamic range of between 2x and 4x. Useful but not dramatic.

3. **Two or more types of particle**
   For a low limit of detection we want a high affinity antibody and lots of it. Whereas for a high upper limit of detection we want a low affinity antibody with lots of particles (> number of antigens to be detected to avoid the hook effect).

   By placing the high affinity antibody on large diameter particles, with a large surface area, we get a high rate of agglutination at low antigen concentrations and because the agglutinated particles are large they generate a large optical signal.

   However, if we use a large number of these particles in an attempt to measure high antigen concentrations then we would have a poor dynamic range as we would end up with a high blank signal and very high optical attenuation. Instead we can use a lower affinity antibody on small particles present in much higher numbers, providing a good upper limit of detection but a poor lower limit of detection.

   Combining the two particles together provides an assay with a larger dynamic range in a single reaction mix. The improvement in dynamic range can be up to 10x that of the simple assay with a single type of particle. Roche has used this technique on its Cobas system[5].

4. **Multiple path lengths**
   The use of different path lengths can also help to extend the dynamic range. At low concentrations a longer path length is required, whilst at high concentrations a short path length is better. This primarily allows us to extend the upper limit of detection rather than the lower limit because the attenuation of the blank signal increases with path length, also this sets the background above which we must detect the antigen.

5. **Gold nanoparticles**
   Latex particles do not scatter light strongly as they have a refractive index close to that of water. Therefore at low antigen concentrations the optical signal generated is very small. This can be enhanced by using particles of high refractive

index such as gold nanoparticles, in a similar manner to lateral flow assays. The aggregation of gold nanoparticles results in a new absorption band at a longer wavelength than that of the dispersed particles which can be used to monitor agglutination. In addition the broadening of the absorption also allows ratiometric measurements to be made, helping to correct for other background influences in the measured concentration.

6. Dynamic light scattering

Dynamic light scattering is a technique that measures the size of particles by looking at how quickly the particles move due to Brownian motion – this can be done in the time or frequency domain. It is often performed by illuminating the sample with a laser and calculating the autocorrelation function of the speckle pattern. In immuno-agglutination this provides a sensitive method of detecting the conjugation of two or more particles and the use of frequency discrimination helps to separate small signals from the background – an advantage that we do not have in simple turbidimetry. In this way Liu et al[6] demonstrated detection of PSA at 5pM using gold nanoparticles – in this case the gold nanoparticles provide strong scattering as described above.

7. Multi-angle nephelometry

Nephelometry measures the light scattered through one or more angles and is potentially more sensitive than turbidimetry. It offers advantages for the detection of low concentrations because in clean samples there is not a large background signal as there is for turbidimetry. In practice, in real time patient samples, there there are other sources of scattering in addition to the immune labelled particles and significant sensitivity improvements have not been obtained in clinical instruments for end point assays. However the use of multi-angle nephelometry is interesting for extending sensitivity and dynamic range as the magnitude and angle of scattering changes with particle size.

8. Go digital

We have seen digital techniques applied to droplet PCR and immuoassays (SIMOA from Quanterix). These techniques can enhance assay sensitivity through the confinement of the signal generation to small fluidic volumes. We can achieve the same benefit in immunoturbidimetry by only measuring the scattering from a small volume and detecting the particles as they flow through, in this way each particle has a large influence on the signal. The individual counting of events (ones and zeros) then allows averages to be calculated. The drawback is that at low analyte concentrations we need to measure many particles to detect a sufficient number of positives to reduce the CV to a clinical acceptable level, a concept that will be familiar to many from digital PCR.

Immunoturbidimetry allows for a relatively simple optical set-up, however is non-linear and this limits the dynamic range that can be acheived

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Conclusion

Single pot assays are very attractive for PoC assays as they remove the need for complex fluid handling and expensive plasticware. Immunoturbiditimetry is a mature technology that meets the practical requirements of a PoC setting. It is, however, limited by its dynamic range and sensitivity. There are, a number of adaptations that can be made that keep the advantages of immunoturbidimetry whilst allowing its range to be extended to clinically useful analytes in the PoC setting. Following these approaches could allow turbidimetry to indeed provide a low-cost, simple solution for immunoassays in the PoC environment.

About the author

Dr. Nick Collier is CTO at global technology & product development company Sagentia and is a keen follower of innovations in science and technology. With a background in Physics and a PhD in semiconductor physics and device fabrication from Cambridge University, Nick has spent his career translating science into robust product designs. Working across the medical, FMCG and industrial sectors he has a depth of expertise in areas such as sensors, actuators and fluidics.

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[1] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/83169
[2] https://www.procalcitonin.com/pct-assays/advia-centaur-brahms-pct.html#correlation-centaur-xp
[3] https://www.ekfdiagnostics.com/procalcitonin.html
[4] http://www.diazyme.com/websites/diazyme/images/products/pdf/data_sheets/MK099-PCT-Flyer.pdf
[5] https://diagnostics.roche.com/us/en/products/systems/cobas_-6000-analyzer-series.html
[6] A One-Step Homogeneous Immunoassay for Cancer Biomarker Detection Using Gold Nanoparticle Probes Coupled with Dynamic Light Scattering Xiong Liu, Qiu Dai, Lauren Austin, Janelle Coutts, Genevieve Knowles, Jianhua Zou, Hui Chen, and Qun Huo*