

# Painless Particles®

Global Newsletter  
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**Bangs Laboratories, Inc.**

Now including Flow Cytometry Standards Corp.

**B E A D S ● A B O V E T H E R E S T™**

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## ISO-9002 Certification



BLI has been certified by TRA Certification as having a Registered Quality System—ISO 9002.

## Visit our New (since February 19) Website

### Bargain

### Bangs ^ Beads & Custom Overstock

See our online list for regular BLI beads (at [www.bangslabs.com/products/bangs/guide.php](http://www.bangslabs.com/products/bangs/guide.php)) for special prices on small quantities of end-of-run, "close-outs", or left-over lots of our microspheres – all sizes, colors, and "flavors". And call us for Custom Overstock specials on flow cytometry beads. Both lists are updated at least monthly, so check back regularly for special deals.

## FCSC + BLI = First Anniversary

One year ago, on May 11, we acquired this flow cytometry standards business. We moved all the equipment, records, raw materials, and product inventory – everything except the people – from Puerto Rico to Indianapolis. Everything went remarkably well; we have had a very successful first year; and *the honeymoon ain't over yet!*

Former customers, who might have been apprehensive about whether the new kids on the block could make products or serve customers as well as FCSC, have been delighted (so they say). In their eyes, we are doing an even better job than before. Apparently, the BLI reputation for quality and service, combined with the excellent products from Puerto Rico, has resulted in some really satisfied customers! So, if you were waiting for any reassurance, come on back!

We proved that we can manufacture improved quality products and are working to bring all FCSC processes under our ISO-9002 quality system this fall, during our annual ISO audit.

We are now setting up a global distributor network wherever flow cytometry is becoming an important research and clinical analytical method. Meanwhile, you can get standards from us.

In this past year, we also found many opportunities for addition of new products to the product line. One example is the soon-to-be-released new product as announced by Nathan Foushee:

### Four-Color QC3 Reference Standard

Do you currently use our **QC3™** multi-color reference standards for instrument set-up or daily **Quality Control**, but wish they were available in a four-color format? If so, **Quit Complaining!** The new four-color **QC3** beads (Cat #844) will soon be available. This **Quintessentially Colorful** product consists of microsphere populations of two different sizes. One is labeled with FITC and PE fluorochromes, while the other is labeled with Cy5 and APC. The two-size platform allows the beads to be run together in one tube, but analyzed separately to eliminate fluorescence "carry-over" between channels. By running only the bead corresponding to the channels in use on a given day, reagents may be conserved (and you will look **Quite Cost-conscious** to the lab manager). The four-color kit will also be available with a certified blank in the popular **QC Windows™** format (Cat #848). (*Well, that boy is certainly enthusiastic, isn't he?*)

## AACC July 31-33 (August 0-2) – Chicago

OK, it's really July 31 - August 2, but putting a couple of extra days in July seemed like more fun. Anyway, we'll be there in force. Most of The Bangs Gang will attend – for a day or more. It's a great way for all to learn about our industry, catch up on the latest technical developments, meet our customers, and learn how you use our products. So be sure to stop by our booth to meet your favorite characters (*and I do mean **characters***) from our place.

**July 31 - August 2 (Tue - Thu): Booth 4008 (OEM Section)** Visit us to learn all about our latest stuff, plans for the future, and answers (maybe even the right answers) to all your questions.

**August 1 (Wed), 9:20 AM: Room S402A – OEM Lecture Series** Nathan Foushee will deliver our presentation, "*QuantumPlex™ Beads Provide the Ideal Platform for a Variety of Multiplexing Applications.*" We were proud to be invited to participate for the 6th consecutive year at this very popular series (up to 100 people at each lecture). *No, they don't let just anybody speak!*

## Cool Applications of Beads

### Quick titer-SQ (Mouse IgG Assay Test Kit)

❖ A new product from Micratech, LLC, of Indianapolis is a semiquantitative strip test which detects IgG's, IgA's, and IgM's in ascites, cell culture, and bioreactor production/purification. It complements their earlier Quick Type-M murine mAb isotyping test kit. (micratech@macconnect.com or www.emicratech.com)

### PickPen (Magnetic Particle Transfer Device)

❖ A convenient, new, handheld device for handling >1µm magnetic beads – like a transfer pipette for beads – with disposable tips. From Bio-Nobile (Turku, Finland). Check it out at www.bio-nobile.com.

## The Bangs Gang

### More News of our Employees – yeah, we're growing!

**Denise Beemster** began as our QC Tech in February. She came to us with a BS in Chemistry from Rose-Hulman Institute of Technology. Since her arrival, she has contributed greatly to our company as an analytical chemist. We expect her to contribute even more in the future.

**Kate Edwards** recently joined the *growing* Customer Service Department, working with Teresa and Mandy to help fill your "particular" needs. She has a strong chemistry/medical background and a great smile. You'll be able to *hear* her smile when you call.



"But don't you see, Gershon - if the particle is too small and too short-lived to detect, we can't just take it on faith that you've discovered it."

**Sharmi Isaiah** has moved from quality control to ProActive coated beads production. This is a promotion for her. People grow up fast around here.

We still have way more women than men at BLI and we guys are feeling discriminated against. Now, don't you feel sorry for us guys?

## P(articles)<sub>2</sub> = Particle Articles

❖ "Microparticle enhanced light scattering assay and microparticle reagents therefor," Eda, S, Kaufman, JH (Roche). *Eur Pat. Appl*, EP 0 898 169 A2 (Pub. Feb 24, 1999). Agglutination reagent and assay using a mixture of two different 30-600nm diameter beads: strongly scattering beads with highly reactive "binding partner" plus weakly scattering beads with low reactivity "binding partner."

❖ "Disruption of the Streptavidin Interaction with Biotinylated Nucleic Acid Probes by 2-Mercaptoethanol", Famulok, *et al.*, *Biotechniques* **26**, 249-254 (February 1999). While the strength of the streptavidin-biotin is advantageous in most applications, sometimes, as when using biotin as an affinity tag for the purification of biological molecules, disrupting the biotin/streptavidin interaction is desirable. This reference offers a protocol for recovering a biotinylated tag by elution with 2-Mercaptoethanol at elevated temperatures. The advantage of this approach over past attempts: minimal damage done to the streptavidin by such treatment.

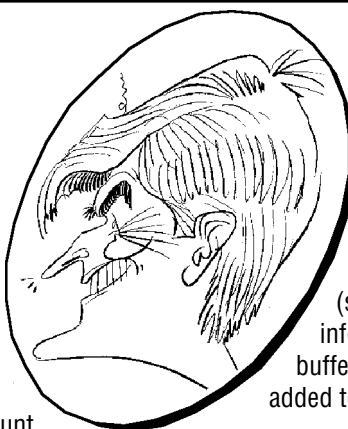
## The Latex Course™ 2001

### A Good News/Bad News Situation

**Bad News:** You missed attending the 14th running of our ever-popular annual course in San Diego April 30 - May 2. We assembled 10 international experts who spoke to the 75 attendees. The course regularly attracts folks who return for a refresher and an annual update on new developments.

**Good News:** We still have a few copies of the >500 page course book, "Designing Microsphere-Based Tests and Assays," which you can purchase for \$350 + shipping. For details of speakers, their biographies, and topics, see our website at www.bangslabs.com/support/latex.php and ask about purchase details. And you'd better hurry – only a few copies are left. (We've sold several since May 2.)

← **No need to "take it on faith" at Bangs Labs.** We will help you discover the particles (beads or microspheres) for your application. Whatever you need: No particles too big or too small here! (Cartoon reprinted with special permission from Sidney Harris.)



## Ask "The Particle Doctor<sup>®</sup>"

### Measuring Solids Content

**Q** : In the process of cleaning microspheres, it seems likely that you will change the solids concentration and lose a fair fraction of particles - especially with small amounts of latex. How do we determine the solids concentration after washing?

**A** : First, we agree that it is important to know the amount of particles you have at any stage of your process - to know the weight of particles per mL, so they can be handled conveniently, and to control your process for addition of the proper amount of wash solution and coating materials. Second, you *will* lose some particles in washing - more or less, depending on which kind of particles you have. "Less" if you are working with monodispersed size microspheres which should all behave the same. While there will always be some losses in transferring things, there won't be too many losses unless the particles are caught in filters. More will be lost if you are working with smaller samples and smaller particles (harder to handle). Losses will also depend on your cleaning process. (*Of course, we won't mind if you want to buy more microspheres to ensure that you have enough.*)

The only reliable way that we know of to measure solids content is by **loss on drying**. We have found good reproducibility and agreement with others' measurements by using as little as 100µL of a well-dispersed (completely resuspended as single particles) suspension at ~10% solids (100 mL at 10% solids = 10 mg which requires a pretty good balance). If solids content is ~1%, then it will certainly take more sample to get good measurable solids. This method may be poorer for low % solids, but it works and it's *only* method.

We shouldn't mention any *bad* ideas, but occasionally somebody tries to use a spectrophotometer to measure solids content by measuring the turbidity of particle dispersions. This is a *bad* idea because turbidity depends on particle size and degree of dispersion, *as well as on particle concentration*. Thus, the absorbance of a 1% solids dispersion of *single* microspheres will be significantly different than 1% solids suspension of *doublets*. And, 1% of 0.2µm microspheres will be very different from 1% of 0.8µm microspheres. Also, everything else in the aqueous phase and on the beads will influence the absorbance. This is a really dangerous method - like skiing out of bounds - you are really in avalanche territory!

It **might** be possible to devise a method whereby microsphere solids and water are dissolved in some solvent (maybe DMF?) and measured in a spectrophotometer at a wavelength sensitive to polystyrene. (I do not know of such a method, I'm only "*composing at the keyboard.*")

### How to Dilute Beads

**Q** : I have purchased BLI microspheres both dry and in suspension. Please advise how to dilute a suspension of microspheres and how to suspend the powdered microspheres; especially, what solutions

are needed?

**A** : Microspheres may be diluted using the buffer they come in (often DI water), or the buffer of choice, following centrifugation or other separation method (see our TechNote 203, *Washing Microspheres*, for more information regarding separation methods). Additional buffer may be added directly to the suspension, or may be added to the pellet following centrifugation.

To suspend dry microspheres, add the buffer of choice and mix (e.g., using an end-over-end mixer, roller, vortexer or sonicator [very carefully]). Surfactant may be added if aggregation is observed - see our TechNote 202, *Microsphere Aggregation*, for details. The duration of the mixing process will depend upon the size and amount of microspheres, i.e. anywhere from a few minutes to a few hours of mixing may be required. You may wish to periodically check the progress of the suspension, i.e., for the presence of aggregates, through microscopy.

### Charge Reversal on Silica

**Q** : Your TechNote 104, *Silica Microspheres*, says it's possible to reverse the charge of silica (from - to +) to adsorb negatively charged DNA. Do you have any references?

**A** : Well, it turns out that Leigh Bangs (who suggested rinsing clean silica in a 0.1-1 M CaCl<sub>2</sub> solution) hasn't been lying to us all these years. Here is an actual reference: Romanowski, G., et al. 1991. Adsorption of plasmid DNA to mineral surfaces and protection against DNase. *Appl Environ Microbiol*, 57(4):1057-1061. Mg<sup>++</sup> or Ca<sup>++</sup> were 100X better than Na<sup>+</sup>, K<sup>+</sup>, or NH<sub>4</sub><sup>+</sup> in the adsorption of plasmid DNA onto sand, indicating a charge-dependent process.

### Mail Bonding (Subscribers "do the 'write' thing!")

- ✦ "Thanks you for sending me this amazing and useful publication." (GD, Parma, Italy) We're happy to serve you. Please write often.
- ✦ "I would love to work for people like you, instead of the filthy rats who pay me to grow prematurely grey today." (Name & place withheld) It sounds as if you are having a bad day. Good luck!
- ✦ "Your customer service has always been excellent. Keep up the good work." (AP, Columbia) Thanks for the kind words.
- ✦ "You have a great site! I really enjoy your company's philosophy. You're obviously having fun and it looks like you're doing well. I suspect you're hauling in the dough! Go for it!" (GM, Stillwater) It's fun and we're doing some good, I hope. Rest is up to you!
- ✦ "You guys really kick ass!" (Nick, Los Angeles) Yeah, we be beads!

**"Inanimate objects are classified scientifically in three major categories  
those that don't work, those that break down, and those that get lost." – Russell Baker**

**Technical References – See our website ([www.bangslabs.com](http://www.bangslabs.com)) for "downloadable" TechNotes and Product Data Sheets or ask for copies by mail or fax. We continually update and add new TechNotes and Product Data Sheets to our website.**

### Product-Specific TechNotes:

101. **ProActive® Microspheres** – Handling tips plus protocols for streptavidin, Protein A, and goat anti-Mouse coated microspheres.
102. **Magnetic Microparticles** – Data + handling tips for >11 varieties of superparamagnetic particles; COOH and NH<sub>2</sub>-modified, classical, encapsulated (low surface iron content), etc.
103. **Fluorescent/Dyed Microspheres** – Applications, fluorescence spectra, and product descriptions. Includes confocal microscopy standards.
104. **Silica Microspheres** – For immunoassays, nucleic acid capture, velocimetry (LDV, PIV), flat panel display spacers, and others.
105. **Microsphere Size Standards** – Data for 9 sizes (0.2-20µm), available singly or in kits, with certificates of analysis.
106. **Confocal Standards** – Using our three, bright, single-label 63nm fluorescent beads in confocal microscopy.

### Handling-Specific TechNotes:

201. **Working with Microspheres** – Choosing, cleaning, characterizing, coating beads, etc.
202. **Microsphere Aggregation** – Preventing, detecting, and reversing aggregation. Chemicals and equipment sources.
203. **Washing Microspheres** – Variety of methods for cleaning microspheres; advantages/disadvantages of methods; suppliers of equipment.
204. **Adsorption to Microspheres** – Adsorbing protein onto particles; use of "surface diluents" (blockers); recipes and references.
205. **Covalent Coupling** – Chemical attachment of proteins, nucleic acids, etc. to various types of surface-functionalized microspheres; recipes for buffers, blockers; miscellaneous coupling ideas, vendor information, and references.
206. **Equations** – For calculating particles/mL, area/g, "parking area", settling velocity @ 1G and in centrifuge, etc.
207. **ProActive® Streptavidin Coated Microspheres and Their Binding Capacity for Biotin and Biotinylated Oligonucleotides** – K Turner, 2000 AACCC OEM Lecture Slides.
208. **Microsphere Sizing** – Various manual and automated methods are described and discussed, with references and supplier list.

### Application-Specific TechNotes:

301. **Immunological Applications** – Review of commercial applications of microspheres.
302. **Molecular Biology** – Overview of purification and solid phase separation methods.
303. **Lateral Flow Tests** – Putting dyed particles on membranes so they will move properly.
304. **Light-Scattering Assays** – Turbidimetric and nephelometric applications of microspheres.

### Reprints:

401. **Estapor® "Microspheres" booklet** – 1995 revision: Information on fluorescents, encapsulated and narrow magnetics, nanoparticles (<50nm), NIST-traceable standards; many handling tips; >60 references.
402. **Microspheres, part 1: Selection, cleaning, and characterization, and part 2: Ligand attachment and test formulation** – LB Bangs & Mary Meza, *IVD Technology (in Medical Device & Diagnostic Industry)*, **17**, #3, 18-26, March, and #4, 20-26, April, 1995. (Note that you can download these papers at the IVDT website: [www.devicelink.com/ivdt/archive/95/03/009.html](http://www.devicelink.com/ivdt/archive/95/03/009.html) and [.../95/04/006.html](http://.../95/04/006.html)).
403. **New Developments in Particle-Based Immunoassays** – Leigh B. Bangs, *Pure & Appl. Chem.*, **68**, #10, 1873-1879 (1996). Review of 40 years of diagnostic uses of microspheres – from LATs to biosensors.
405. **Applications of Magnetic Particles in Immunoassays** – Mary Meza, Ch. 22 (pp. 303-309) in *Scientific and Clinical Applications of Magnetic Carriers*, U. Häfeli, *et al*, Eds., Plenum Press, New York, 1997.
406. **Measuring Microsphere Binding Capacity** – JM Duffy, JV Wall, MB Meza, LJ Janski, *IVD Technology*, **4**, #7, 28-34 (1988). (No reprints are available; you can download from our website.)
407. **Bead-based HTS Applications in Drug Discovery** – MB Meza, *Drug Discovery Today: HTS Supplement*, **1**, #1, 38-41 (2000).

**Flow Cytometry Standards?** See the FCSC portion of our website for lots of technical information about flow cytometry standardization in general and our new flow cytometry standards products in particular.

**Ask us to help find...** cleaning equipment, big beads, slides, membranes for strip tests, etc. (Or see the "Hot Links" page at our website.)

**Free Literature for you!** What information do *you* need? We freely share our library: >1000 papers about microspheres, cross referenced, so we can search for types of particles, coupling methods, uses, author, etc. New papers are added as we get them.  
**Help from you?** Please tell *us* about good papers which we should have as you find them. And please send us any good bead art that you find – photos, drawings, etc. showing microspheres or their applications.