A multiple reaction monitoring liquid chromatography method with tandem mass spectrometric detection for quantitation of Staphylococcus aureus via phage amplification detection is described. This phage amplification detection method enables rapid and accurate quantitation of viable S. aureus by detecting an amplified capsid protein from a specific phage. A known amount of metabolically labeled 15N reference bacteriophage, utilized as the input phage and as the internal standard for quantitation, was spiked into S. aureus samples. Following a 2-h incubation, the sample was subjected to a 3-min rapid trypsin digest and analyzed by high-throughput liquid chromatography tandem mass spectrometric detection targeting peptides unique to both the 15N (input phage) and 14N (progeny phage) capsid proteins. Quantitation was achieved by comparing peak areas of target peptides from the metabolically labeled 15N bacteriophage peptide internal standard with that of the wild-type 14N peptides that were produced by phage amplification and subsequent digestion when the host bacteria was present. This approach is based on the fact that a labeled species differs from the unlabeled one in terms of its mass but exhibits almost identical chemical properties such as ion yields and retention times. A 6-point calibration curve for S. aureus concentration was constructed with standards ranging from 5.0 × 10⁴ colony forming units (CFU) ml⁻¹ to 2.0 × 10⁶ CFU ml⁻¹, with the 15N reference phage spiked at a concentration of 1.0 × 10⁸ plaque forming units (PFU) ml⁻¹. Amplification with 15N bacteriophage coupled with LC-MS/MS detection offers speed (3 h total analysis time), sensitivity (LOD: < 5.0 × 10⁴ CFU ml⁻¹), accuracy, and precision for quantitation of S. aureus. Molecular & Cellular Proteomics 11: 10.1074/mcp.M111.012849, 1–11, 2012.

Staphylococcus aureus (S. aureus) is a versatile pathogenic bacterium (1) responsible for a significant number of healthcare-associated and community-acquired infections (2). It causes a broad spectrum of infections ranging from acute to chronic disease (3, 4) and is a common etiological agent of opportunistic infections. An increasing prevalence of antibiotic resistant S. aureus strains are emerging, posing an even greater threat to the general public worldwide (5). For these reasons, the development and improvement of diagnostic methods that allow rapid identification and quantitation of this bacterium are highly critical (2).

Several methods are available for definitive identification of S. aureus. Traditional identification through plate-culture requires 2–3 days, can require subculturing or biochemical analysis (6), and necessitates blood sample volumes difficult to obtain in pediatric patients (7). Molecular methods, such as polymerase chain reaction, reverse transcription-polymerase chain reaction, and quantitative reverse transcription-polymerase chain reaction (PCR, RT-PCR, and qRT-PCR) are primarily based on particular genes specific to S. aureus (2) and offer the most sensitive measurements in the least amount of time, with the least amount of sample, but can be prone to ambiguous results that can only be resolved through sample cultivation (5). Additionally PCR is unable to distinguish dead bacteria from live cells, and the required reagents are relatively costly. Gene probe assays are promising in that they offer simple, rapid, and sensitive measurements and are lower in cost than PCR techniques (2, 6). Several rapid methods (culture-based and molecular-based screening methods) for S. aureus are also available, allowing diagnostics within hours of collection time; however these tests can be costly and the majority yield qualitative, not quantitative results (8–10).

Isotope labeling MS has long been used for quantitation of small molecules in a variety of matrices (11). More recently, MS-based stable isotope tagging of proteins and peptides followed by MS/MS experiments (12) has emerged as an important tool in quantitative proteomic experiments (13–19). Quantitation is achieved by adding a known amount of stable isotope-labeled protein or peptide to a sample as an internal standard and comparing instrument response to an unlabeled
counterpart. Because species tagged with heavy isotopes differ from the unlabeled light ones in terms of their mass but exhibit almost identical chemical properties such as ion yields and retention times (20), ionic signals from tagged ion pairs can be accurately compared independently from instrument response. Labeling strategies include the use of chemical reactions to introduce an isotopic or isobaric tag at specific functional groups on polypeptides (21–24), metabolic isotope labeling using heavy amino acids (25–29), and methods that introduce stable isotopes via enzymatic reactions (30, 31, 32). Each of these methods has specific strengths and weaknesses (32); however, metabolic incorporation of stable isotopes in whole organisms using cell culturing in heavy media is attractive in that it allows labels to be introduced at the earliest time point possible, during protein synthesis, and offers the most comprehensive way to cover proteomes completely (33, 34). Heavy isotopes, such as $^{15}$N and $^{13}$C, in nutrients fed to organisms during growth result in incorporation of heavy labels into all proteins over the course of doubling (35). Labeled controls and unlabeled samples are then combined prior to sample preparation, providing an internal control to reduce variability in the comparison of two proteomes (36).

The use of phages for bacterial detection is well documented (37). Bacteriophages are specific to their target host, self-replicate, have extensive shelf lives, are inexpensive (38), and infect only metabolically active cells (39). Bacteriophage amplification technology has emerged as a means of rapid bacterial detection, using modern protein analytical techniques to monitor changes in sample bacteriophage concentration (40–43). Standard phage amplification detection (PAD)$^{1}$ techniques are highly specific, but the progeny phage produced through infection cannot be differentiated from the input parent phage initially added to the sample. For this reason, standard PAD techniques must use low-level concentrations of input phage, well below the detection limit of the detection device, thus prolonging incubation time prior to analysis, decreasing analysis frequency, increasing detection limits, and potentially increasing the probability of false positive results. To overcome these limitations, we have recently developed a rapid $^{15}$N PAD approach for analysis of intact phage proteins as a means of detecting S. aureus via top-down matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (44). Although this $^{15}$N PAD MALDI-TOF MS method has proven successful for detecting viable S. aureus via amplified phage capsid proteins, it is unable to quantify S. aureus concentration because of the inherent problems in obtaining quantitative MALDI results.

Here, we present a “bottom-up” approach that combines PAD, stable isotope metabolic labeling, and liquid chromatography–multiple reaction monitoring tandem mass spectrometry (LC-MRM MS/MS) to quantify Staphylococcal bacteriophage 53 peptides from a phage capsid head protein, which when present, are indicative of the concentrations of viable host S. aureus bacteria (ATCC 27694). We show that our $^{15}$N PAD LC-MS/MS method offers good accuracy, precision, and sensitivity for high-throughput quantitation of viable S. aureus. To our knowledge, this is the first study employing $^{15}$N PAD combined with LC-MS/MS to quantify target bacteria.

**EXPERIMENTAL PROCEDURES**

*S. aureus and Bacteriophage 53—Stock cultures of lyophilized S. aureus (ATCC 27694) and bacteriophage 53 (ATCC 27694-B1) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). All microbiological procedures were performed in a U.S. biological safety level 2 (BSL2) facility following standard biosafety protocols. $^{15}$N bacteriophage stocks were generated by combining 500 $\mu$l of dense ATCC 27694 culture with 500 $\mu$l of wild-type bacteriophage 53 ($1.0 \times 10^{6}$ PFU ml$^{-1}$) in a test tube, pouring the contents onto a tryptic soy agar plate, and incubating the plate overnight at 37 °C. Following incubation, the plate’s content was collected into a 50-ml conical tube by washing with tryptic soy broth (TSB) (Bacto™ TSB, BD Diagnostics, Franklin, NJ), scraping with a sterile plastic loop, and aspirating with a pipette. The conical tube was then centrifuged for 20 min at 3500 rpm to pellet out the debris, and the supernatant was filtered through an Autovial 0.2 $\mu$m polyvinylidene difluoride membrane syringeless filter device (Whatman, Inc, Clifton, NJ) to remove extraneous debris. The final phage filtrate was quantified using a traditional plaque assay (45).

*Biosynthetic Production of $^{15}$N S. aureus and $^{15}$N Bacteriophage 53—Stock cultures of lyophilized S. aureus and $^{15}$N S. aureus cells were grown in Bioexpress Cell Growth Media U-$^{15}$N, 98% (Cambridge Isotope Laboratories, Inc., Andover, MA) according to manufacturer’s instructions with only minor modifications. A 1-ml sample of $^{15}$N cell growth media was added to 9 ml of 0.1 M phosphate buffered saline (PBS), inoculated with 500 $\mu$l of S. aureus ATCC 27694, and allowed to grow overnight at 37 °C. Following overnight culture, the same procedure was repeated with the exception of inoculating the fresh $^{15}$N cell growth media with 500 $\mu$l of the dense overnight bacterial growth. This double-round inoculation of ATCC 27684 with $^{15}$N cell growth media ensured maximum labeling efficiency. Following the second overnight incubation, the dense $^{15}$N labeled bacteria were combined with 500 $\mu$l of wild-type bacteriophage ($1.0 \times 10^{6}$ PFU ml$^{-1}$) and added to a $^{15}$N-labeled agar plate. The $^{15}$N-labeled agar plates were made by combining 10 ml of $^{15}$N cell growth media, 90 ml of de-ionized water, and 1.5 grams of agar. The solution was autoclaved for 60 min and poured into sterile Petri dishes. After allowing the $^{15}$N S. aureus and $^{15}$N bacteriophage to incubate overnight on the $^{15}$N-labeled agar plate, the plate’s contents was collected into a 50-ml conical tube by washing with 5 ml TSB, scraping with a sterile plastic loop, and aspirating with a pipette. The conical tube was then centrifuged for 20 min at 3500 rpm to pellet out the debris, and the supernatant was filtered through an Autovial 0.2 $\mu$m polyvinylidene difluoride membrane syringeless filter device to remove extraneous debris. The $^{15}$N-labeled bacteriophages were quantified using a standard plaque assay (45). Phage stock suspensions ($2.1 \times 10^{6}$ PFU ml$^{-1}$) were stored at 4 °C for further analyses.

**Nano-LC-MS/MS—**Protein identification was performed as previously reported (44). Briefly, 5-$\mu$l tryptic digests of the $^{15}$N and $^{15}$N phage stock solutions were analyzed using a Waters NanoAquity
liquid chromatography system (Waters Corporation, Milford, MA) utilizing a 100 μm × 100 mm BEH130 C18 analytical column (1.7 μm particle size) (Waters Corporation). The aqueous mobile phase (A) consisted of HPLC-grade water with 0.2% formic acid (FA) and 0.005% trifluoroacetic acid, whereas the organic phase (B) was acetonitrile with 0.2% FA and 0.005% trifluoroacetic acid. A gradient profile was used at a flow rate of 400 nL min⁻¹. Initially, the mobile phase consisted of 5% B and 95% A. After 5 min, the gradient was ramped to 30% B over the next 100 min, continuing up to 90% B over the next 5 min and holding for 2 min. After 112 min total run time, the gradient was returned to 5% B and 95% A for the next 20 min to equilibrate the column to initial conditions. The total run time was 132 min.

The column eluent was introduced into a Thermo Scientific LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, Waltham, MA) equipped with an electrospray interface. The LTQ-Orbitrap instrument performed data-dependent acquisition of precursor and product ion spectra. The nominal resolution of the Orbitrap analyzer was set at 60,000 with scan range from m/z 200 to 1600. The top nine most intense ions were selected for fragmentation; the selection window was set at m/z = 2. Conventional collision induced dissociation was used for ion activation purposes. Dynamic exclusion was enabled with a repeat count of 1 and an exclusion duration of 120 s. Charge state screening and monoisotopic precursor ion selection were enabled along with charge state rejection, whereby singly charged ions were not selected for MS/MS analysis.

Protein Identification and Target Peptide Determination—Protein identification was performed by matching acquired peptide tandem mass spectra to theoretical digests found in a protein database. Prior to database searching, MS/MS spectra were converted to Mascot generic format by using Mascot Distiller version 2.3.2.0 (Matrix Science, London, UK). Charge state deconvolution and de-isotoping were not performed. Database searching utilized the Mascot version 2.2.0 algorithm (Matrix Science, London, UK) with the following parameters: fragment ion mass tolerance of 0.50 Da, parent ion tolerance of 200 ppm, trypsin enzyme, two missed or nonspecific cleavages permitted, and deamidation of asparagine and oxidation of methionine specified as variable modifications. A database of 13,767,831 entries was generated by extracting sequences from the National Center for Biotechnology (NCBI) protein database (downloaded on 04/22/2011) that contained at least one of the following strings in the entry description; “staphylococcus phage,” “aureus,” “trypsin,” “keratin.” To validate MS/MS-based peptide and protein identification, Scaffold version 3.00.02 (Proteome Software Inc., Portland, OR) with PeptideProphet™ (46) and ProteinProphet™ (47) algorithms was used. Peptide identifications were accepted if they could be established at greater than 95.0% probability as specified by PeptideProphet™. Protein identifications were accepted if they could be established at a 99.0% probability based on ProteinProphet™, and if they were based on at least 3 peptide matches. Proteins containing similar peptides and that could not be differentiated based on MS/MS analysis alone were grouped into a single match.

The peptide sequences SIAQSIEK, LGVILPVTK, and LIYGIPQIEKY were selected for quantitation purposes. The metabolically labeled SIAQSIEK and LGVILPVTK were 10 Da heavier than the naturally occurring peptides, because of incorporation of serine, isoleucine, alanine, glutamic acid, leucine, glycine, valine, proline, and threonine each with a 15N nitrogen count of 1, and glutamine and lysine amino acids with a 15N nitrogen count of 2. Similarly, labeled LIYGIPQIEKY was 14 Da heavier than the naturally-occurring peptide.

Preparation of S. aureus Working Stock and Standard Solutions—To determine the S. aureus concentration (CFU ml⁻¹) and to prepare calibrated standards, serial dilutions of S. aureus concentrated overnight suspensions (working stocks) were made and ab sorbance spectra for each dilution were recorded at 650 nm using a SpectraMax 2 (Molecular Devices, Inc., Sunnyvale, CA) spectrophotometer. The bacterial dilutions were then plated for viable cell counting. After overnight growth at 37 °C, the relationship between absorbance and the CFU ml⁻¹ was graphed and values in the linear range of this graph were used to calculate the concentration of S. aureus working stocks solutions.

For quantitation, an initial absorbance reading was taken of the S. aureus working stock solution and was used as the starting point when making serial dilutions for calibration curves. From the working stock, six 1-ml serial dilutions in TSB were prepared ranging from 5.0 × 10⁶ CFU ml⁻¹ to 2.0 × 10⁴ CFU ml⁻¹. The six 0.5-ml calibration standards, ranging from 5.0 × 10⁶ CFU ml⁻¹ to 2.0 × 10⁴ CFU ml⁻¹, were prepared by adding 50 μl of the corresponding S. aureus dilution, 250 μl of stock 15N phage (2.1 × 10⁸ PFU ml⁻¹), 50 μl of 1 mM magnesium chloride, and 150 μl TSB, to make the final volume, 0.5 ml. The 250 μl 500 ml⁻¹ of stock 15N phage was used as the internal standard (ISTD).

S. aureus Quantification Using 15N PAD LC-MS/MS

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Target Staphylococcal bacteriophage 53 capsid protein peptide sequences and their corresponding \(^{15}\)N metabolically labeled counterparts.

\(|\text{Target peptide} \quad \text{Actual Mass Da} \quad \text{Precursor Ion } m/z \quad \text{Fragment Ion (quantitation)} \quad \text{Fragment Ion (confirmation)} \quad \text{Fragment Ion (confirmation)}||
| SIAQSIEK-\(^{14}\)N | 874.5 | 438.2 (±2) | 675.4 (y6) | 476.3 (y4) | 604.3 (y5) |
| SIAQSIEK-\(^{15}\)N | 884.5 | 443.2 (±2) | 683.4 (y6) | 481.3 (y4) | 611.3 (y5) |
| LGVILPVTK-\(^{14}\)N | 938.6 | 470.3 (±2) | 670.5 (y6) | 557.4 (y5) | 444.3 (y4) |
| LGVILPVTK-\(^{15}\)N | 948.8 | 475.4 (±2) | 677.4 (y6) | 563.3 (y5) | 449.3 (y4) |
| LIYGIPQLEYK-\(^{14}\)N | 1448.8 | 725.4 (±2) | 890.5 (y7) | 1223.7 (y10) | 1060.6 (y9) |
| LIYGIPQLEYK-\(^{15}\)N | 1462.8 | 732.4 (±2) | 899.5 (y7) | 1235.7 (y10) | 1071.6 (y9) |

3 min the gradient was stepped to 80% A and 20% B over the next 7 min. After 10 min the gradient was stepped to 75% A and 25% B over the next 5 min and then held constant for 2 min. After 17 min total the gradient was stepped to 2% A and 98% B for 7 min to clean the column, then stepped to 98% A and 2% B for the next 3 min to begin equilibrating the column to initial conditions. The isocratic gradient for the regeneration column utilized a 50 \(\mu\)l min \(^{-1}\) flow rate and consisted of a constant eluent composition of 98% A and 2% B. The total analysis run time was 28 min.

The column eluent was introduced into a Thermo Scientific Vantage TSQ triple quadrupole tandem mass spectrometer with an electrospray interface (Thermo Scientific, Waltham, MA). The instrument was operated in positive ion multiple reaction monitoring mode. The precursor—fragment transitions were shifted by 14 Da from its unlabeled counterpart. These results were shifted by 10 Da and the LIYGIPQLEYK peptide was shifted by 14 Da from its unlabeled counterpart. These results were expected because SIAQSIEK and LGVILPVTK both have 10 nitrogen atoms whereas LIYGIPQLEYK contains 14 nitrogen atoms. No evidence was seen for unlabeled or partially labeled peptides in the analysis. To evaluate mass spectrometric instrument response for the wild-type phage tryptic peptides of interest, and to establish the analytical limit of detection, twofold dilution series of the wild-type \(^{15}\)N bacteriophage-derived peptides were analyzed using the MRM transitions described in Table I. Typical chromatographic traces for quantitation and confirmation transitions are shown in supplemental Fig. S2. The resulting curves from the dilution series confirmed that the instrument response was proportional to the \(^{15}\)N bacteriophage concentration for the MRM quantitative transitions (supplemental Fig. S3) with a detection limit of \(\approx 3.0 \times 10^5\) PFU ml \(^{-1}\), based upon the observed S/N of at least 500 for the SIAQSIEK quantitative transition (supplemental Fig. S2). This is 100 times more sensitive than the previously reported \(^{15}\)N PAD MALDI method (44).

A \(^{15}\)N PAD experiment was conducted to confirm that the developed MRM instrumental method could detect an increase in progeny phage concentration, thereby implying the presence of \(S.\ aureus\). In this experiment, duplicate samples

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### Table I

| Target peptide | Actual Mass Da | Precursor Ion \(m/z\) | Fragment Ion (quantitation) | Fragment Ion (confirmation) | Fragment Ion (confirmation) |
|----------------|----------------|----------------------|-----------------------------|----------------------------|-----------------------------|
| SIAQSIEK-\(^{14}\)N | 874.5 | 438.2 (±2) | 675.4 (y6) | 476.3 (y4) | 604.3 (y5) |
| SIAQSIEK-\(^{15}\)N | 884.5 | 443.2 (±2) | 683.4 (y6) | 481.3 (y4) | 611.3 (y5) |
| LGVILPVTK-\(^{14}\)N | 938.6 | 470.3 (±2) | 670.5 (y6) | 557.4 (y5) | 444.3 (y4) |
| LGVILPVTK-\(^{15}\)N | 948.8 | 475.4 (±2) | 677.4 (y6) | 563.3 (y5) | 449.3 (y4) |
| LIYGIPQLEYK-\(^{14}\)N | 1448.8 | 725.4 (±2) | 890.5 (y7) | 1223.7 (y10) | 1060.6 (y9) |
| LIYGIPQLEYK-\(^{15}\)N | 1462.8 | 732.4 (±2) | 899.5 (y7) | 1235.7 (y10) | 1071.6 (y9) |
containing $1.0 \times 10^5$ PFU ml$^{-1}$ of $^{15}$N labeled bacteriophage and $2.0 \times 10^6$ CFU ml$^{-1}$ of S. aureus were prepared. Following addition of the labeled bacteriophage, one replicate was immediately filtered, digested, and analyzed (t = 0 h), whereas the second replicate was subjected to a 2-h incubation period at $37 \, ^\circ$C. Fig. 2 shows extracted ion chromatograms for this experiment. Fig. 2A (t = 0 h) shows high signal intensities for each $^{15}$N quantitative transition, whereas instrument response to the native bacteriophage ($^{14}$N progeny) quantitative transition is negligible. Following the 2-h incubation, no significant differences were observed in $^{15}$N signal intensities, however, relative to the $t = 0$ h extracted ion chromatograms (Fig. 2A), a dramatic increase in signal intensity was observed for all three $^{14}$N quantitative transitions (Fig. 2B). Because the only means of generating the $^{14}$N phage peptides is through amplification of the progeny bacteriophage by a viable strain of S. aureus, these results confirm that the $^{15}$N PAD LC-MS/MS method can be used to positively detect the presence of S. aureus. Additionally, to look for background levels of the $^{14}$N phage peptides and to test for interferences, a $t = 0$ h S. aureus control sample was incorporated into each experimental sample set. This control sample was prepared identically to samples for $^{15}$N PAD, with the exception that it was not subjected to the $t = 2$ h phage amplification event. Following sample preparation, this control was immediately filtered, washed, digested, and analyzed by LC-MS/MS. Daily analyses showed no background levels ($< 5.0 \times 10^5$ CFU ml$^{-1}$) of $^{14}$N phage peptides present in any of the unamplified samples, and that there were not interferences to the analysis (Fig. 2A).

The purpose of using $^{15}$N labeled bacteriophage in PAD experiments is twofold. First, to distinguish parent bacteriophage ($^{15}$N labeled input) from progeny bacteriophage ($^{14}$N wild type output) by their mass differences. The use of a heavy phage provides more confidence in the mass spectrometric analyses since the parent and progeny can be differentiated by mass. Second, by using the $^{15}$N labeled phage as an internal standard and a standard growth parameter, the number of bacteria can be quantified. To quantify the bacteria in culture, a high concentration of $^{15}$N bacteriophage must be used to ensure conditions where at least one infective $^{15}$N bacteriophage is attached to each S. aureus bacterium, preventing any further bacterial growth. For this condition to be met, a high multiplicity of infection (MOI); i.e. the ratio of infectious bacteriophage to bacteria, and a sufficiently dense concentration of bacteriophage must exist (55). The number of bacteriophages that infect a given bacterial cell can be calculated from the Poisson distribution, given as:

$$P(n) = m^n e^{-m}/n!$$

where, $P(n)$ is the probability of bacterial cells being infected by n phage, and $m$ is the MOI. Using overnight cultures, our experimentally determined mean bacterial density was found to be $3.6 \times 10^8$ CFU ml$^{-1} \pm 5\%$ over the course of five different days. For the given $^{15}$N bacteriophage concentration of $1 \times 10^5$ PFU ml$^{-1}$ used in this study, the lowest theoretical MOI that would be encountered in the experimental design is 2.77. 

supplemental Fig. S4 plots $P(n)$ versus n for a MOI of 2.77. From the plot it can be seen that $\geq 95\%$ of all bacteria will have at least one infectious bacteriophage attached for a sample of S. aureus at a concentration of the $3.6 \times 10^8$ CFU ml$^{-1}$. Thus, theoretically if concentrations of bacteriophage are kept below $3.6 \times 10^8$ CFU ml$^{-1}$, all bacteria can be assumed phage-infected, leading to accurate quantification. Empirically, as the concentration of viable S. aureus approaches $3.6 \times 10^8$ CFU ml$^{-1}$, the number of live bacteria could be underestimated if high concentrations of dead S. aureus cells, in conjunction with viable S. aureus cells, exist in a given sample, and result in competitive $^{15}$N bacteriophage binding, shifting the MOI lower than 2.77. For the purposes of this quantitative study the dynamic range was $5.0 \times 10^4$ CFU ml$^{-1}$ to $2.0 \times 10^6$ CFU ml$^{-1}$, considerably lower than $3.6 \times 10^8$ CFU ml$^{-1}$, to ensure that $\geq 95\%$ of all (viable or otherwise) bacteria have at least one infectious bacteriophage attached even in the presence of a significantly high concentration of dead S. aureus cells.

Various modeling and experimental studies have been conducted that describe bacteriophage and bacteria proliferation concentration thresholds that must be met for a productive phage infection event (56). To ensure that at high phage concentrations all bacteria are infected immediately after inoculation, various concentrations of bacteria ($1 \times 10^4$ to $1 \times 10^7$ CFU ml$^{-1}$) were inoculated at $1.0 \times 10^9$ PFU ml$^{-1}$ bacteriophage, and allowed to incubate for 15 min. Following incubation, each sample was serially diluted and plated onto tryptic soy agar. After culturing the plates overnight, colonies on each plate were counted and compared against control plates that contained the same bacterial con-
S. aureus Quantification Using $^{15}$N PAD LC-MS/MS

Fig. 2. Liquid chromatography tandem mass spectrometry (LC-MS/MS) extracted ion chromatograms of the quantitative transitions monitored. The chromatograms show a standard solution ($2.0 \times 10^6$ CFU ml$^{-1}$) A, $t = 0$ h; prior to the phage amplification event and B, $t = 2$ h; the end point of the infection. The $t = 0$ h and $t = 2$ h chromatograms are displayed on identical scales based on ion counts.

concentrations without bacteriophage infection. At each bacterial concentration tested, the cultures infected with $1.0 \times 10^9$ PFU ml$^{-1}$ bacteriophage showed no bacterial growth, suggesting that all S. aureus were rapidly infected within the first minutes of phage infection.

Following these preliminary method characterization studies, 6-point calibration curves ranging from $5.0 \times 10^4$ to $2.0 \times 10^6$ CFU ml$^{-1}$ were generated on five different days with three replicate LC-MS/MS injections for each standard. The metabolically $^{15}$N reference phage was spiked at a concentration of $1.0 \times 10^9$ PFU ml$^{-1}$. The mean unlabeled and labeled MRM area ratios for each quantitative transition analyzed were plotted against expected and labeled MRM area ratios for each quantitative transition monitored. The chromatograms show a standard solution ($2.0 \times 10^6$ CFU ml$^{-1}$) A, $t = 0$ h; prior to the phage amplification event and B, $t = 2$ h; the end point of the infection. The $t = 0$ h and $t = 2$ h chromatograms are displayed on identical scales based on ion counts.

deviations (% RSD; (standard deviation/mean)$\times 100$) are reported in Table II. Mean S. aureus concentrations for five replicates spiked at $1.0 \times 10^5$ CFU ml$^{-1}$ and $1.0 \times 10^6$ CFU ml$^{-1}$ levels produced highly reproducible results with % RSDs of ≤ 15%, for all three quantitative transitions, demonstrating the effectiveness of the method. Similarly, interpeptide %RSDs of ≤ 2% and ≤ 9% for $1.0 \times 10^5$ CFU ml$^{-1}$ and $1.0 \times 10^6$ CFU ml$^{-1}$, respectively, show significant agreement among the three transitions used for quantification, indicating that the evaluated peptides did not differ with respect to precision. To ascertain the accuracy of the measurements the experimentally determined S. aureus concentrations were compared against the amounts spiked, as measured by optical density readings that were correlated to bacterial plate counts. Interpeptide accuracies were determined to be 31% and 1% for the $1.0 \times 10^5$ CFU ml$^{-1}$ and $1.0 \times 10^6$ CFU ml$^{-1}$ concentrations, respectively. Although the accuracy of the low-level spike appears to have a slight high bias, CFUs are only an estimate of the number of cells present (57), as the accuracy of the method is dependent on the reference curve obtained via direct plate count and optical density readings. Despite the limitations of plate counting and optical density readings we have rigorously standardized all steps in our $^{15}$N PAD method to control and minimize error in the analyses.

Finally, verification that complete digestion has been achieved is essential for accurately quantifying proteins using MS (58), and to ensure long term stability of the quantification method. Digestion parameters, including incubation time and temperature, amount of trypsin, and amount of acid-labile detergent, were varied and peptide recoveries were determined. Maximum peptide yields were assumed to have been achieved when no further increase in $^{14}$N or $^{15}$N peptide
S. aureus Quantification Using $^{15}$N PAD LC-MS/MS

**Table II**

| Spike concentration (CFU ml$^{-1}$) | intra-peptide concentration (mean ± S.D. $^{15}$N) | intra-peptide concentration (mean ± S.D. %RSD) | inter-peptide concentration (mean ± S.D. %RSD) |
|------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| S. aureus Measurements (CFU ml$^{-1}$) | n = 5 | 9.70 x 10$^{-6}$ ± 1.02 x 10$^{-6}$ (10.7%) | 1.01 x 10$^{-6}$ ± 1.16 x 10$^{-6}$ (11.6%) |
| S. aureus Measurements (CFU ml$^{-1}$) | 1.00 x 10$^{6}$ | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) |
| S. aureus Measurements (CFU ml$^{-1}$) | 1.00 x 10$^{6}$ | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) |
| n = 5 | | | |
| SIAQSIEK | | | |
| LGEILPVTK | | | |
| LIYGIPQLIEYK | | | |

Rapid and accurate detection of pathogenic organisms is crucial to diagnosis, treatment, and prevention of disease. The use of $^{15}$N labeled bacteriophage in PAD experiments allows for greater accuracy in the detection of the phage progeny because the input and output phage are distinguishable by mass. The use of $^{15}$N labeled bacteriophage allows for greater amounts of input phage to be used, which decreases the time of analysis and the use of LC-MS/MS improves detection limits. Additionally, using $^{15}$N labeled bacteriophage is beneficial in that the $^{15}$N labeled phage proteins act as an internal standard allowing rapid, accurate, and sensitive quantitation of S. aureus. The rapid identification and accurate quantification of S. aureus required (1) developing a rapid $^{15}$N phage amplification step that could simultaneously infect all S. aureus cells; (2) developing a rapid and efficient proteolytic digestion method; and (3) employing high-throughput LC-ESI-MS/MS for rapid, sensitive, and specific quantitation. The ability of our method to inoculate with high $^{15}$N-labeled phage titers (higher than the LC-MS/MS detection limit) allows S. aureus cells to be infected simultaneously, permitting S. au-

amounts could be observed. Three specific peptides from different regions of the protein were quantified to ensure complete digestion of the protein and accuracy of the measurements. Three digestion protocols were compared (1) overnight tryptic digestion at 37 °C; (2) 2-h tryptic digestion at 37 °C; and (3) 3-min tryptic digestion at 52 °C. For each protocol, triplicate S. aureus samples at 5.0 x 10$^{5}$ CFU ml$^{-1}$, were subjected to the 2-h phage amplification event, proteolytically digested, and analyzed by LC-ESI-MS/MS. Each sample preparation was analyzed in triplicate. Table III shows the comparison of the different digestion conditions. The number of live S. aureus cells obtained from three independent analyses of a standard solution of 5.0 x 10$^{5}$ CFU ml$^{-1}$ following phage infection were determined to be 5.0 x 10$^{5}$ CFU ml$^{-1}$, 4.7 x 10$^{5}$ CFU ml$^{-1}$, and 5.6 x 10$^{5}$ CFU ml$^{-1}$ for the 3 min/52 °C, 2 h/37 °C and 18 h/37 °C digestion protocols, respectively, indicating that all digestion techniques yielded good precision and accuracy (Table III). Means for the three quantitative peptide transitions, at each digest condition, were reproducible with %RSDs of $\leq$ 13%. Interpeptide agreement at each digest condition resulted in %RSDs of $\leq$ 5%, indicating that each protocol was robust and suitable for the peptides evaluated. The inter-peptide accuracy of the 3 min $^{15}$N PAD MS method was calculated to be 100%, indicating that the rapid 3-min digest incubated at 52 °C was a good, rapid alternative to the longer traditional tryptic digestion methods that use 37 °C incubation temperature and 2–18 h digestion times. Furthermore, the accuracy of the method and the good agreement between values obtained independently on the three peptides suggests that all three digestion techniques yielded complete digestion of the major capsid protein of bacteriophage 53.

**DISCUSSION**

Rapid and accurate detection of pathogenic organisms is crucial to diagnosis, treatment, and prevention of disease. The use of $^{15}$N labeled bacteriophage in PAD experiments allows for greater accuracy in the detection of the phage progeny because the input and output phage are distinguishable by mass. The use of $^{15}$N labeled bacteriophage allows for greater amounts of input phage to be used, which decreases the time of analysis and the use of LC-MS/MS improves detection limits. Additionally, using $^{15}$N labeled bacteriophage is beneficial in that the $^{15}$N labeled phage proteins act as an internal standard allowing rapid, accurate, and sensitive quantitation of S. aureus. The rapid identification and accurate quantification of S. aureus required (1) developing a rapid $^{15}$N phage amplification step that could simultaneously infect all S. aureus cells; (2) developing a rapid and efficient proteolytic digestion method; and (3) employing high-throughput LC-ESI-MS/MS for rapid, sensitive, and specific quantitation. The ability of our method to inoculate with high $^{15}$N-labeled phage titers (higher than the LC-MS/MS detection limit) allows S. aureus cells to be infected simultaneously, permitting S. au-

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**Table III**

| Spike concentration (CFU ml$^{-1}$) | intra-peptide concentration (mean ± S.D. %RSD) | inter-peptide concentration (mean ± S.D. %RSD) |
|------------------------------------|-----------------------------------------------|-----------------------------------------------|
| S. aureus Measurements (CFU ml$^{-1}$) | n = 5 | 1.30 x 10$^{-6}$ ± 1.16 x 10$^{-6}$ (8.9%) | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) |
| S. aureus Measurements (CFU ml$^{-1}$) | 1.00 x 10$^{6}$ | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) |
| S. aureus Measurements (CFU ml$^{-1}$) | 1.00 x 10$^{6}$ | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) | 1.00 x 10$^{-6}$ ± 1.00 x 10$^{-6}$ (0%) |
| n = 5 | | | |
Verifying completeness of digestion for the SIAQSIEK, LGVILPVTK, and LIYGIPQLIEYK quantitative peptide transitions. For \(^{15}\text{N}\) PAD MS measurements, \(5.0 \times 10^5\) CFU ml\(^{-1}\) of spiked S. aureus was subjected to phage amplification, tryptic digest, and LC-MS/MS analysis. Triplicate results for each preparation are shown and intra-peptide and inter-peptide means (CFU ml\(^{-1}\) tested), standard deviations, and percent relative standard deviations (% RSDs) for \(n = 3\) sample preparations is reported. S.D. = standard deviation; % RSD = % relative standard deviation.

### 3-min Digestion Protocol (\(5 \times 10^5\) CFU ml\(^{-1}\) spiked S. aureus)

|       | 3-min \(^{15}\text{N}\) PAD Digest 1 | 3-min \(^{15}\text{N}\) PAD Digest 2 | 3-min \(^{15}\text{N}\) PAD Digest 3 | 3-min \(^{15}\text{N}\) PAD Digest Intra-peptide (mean ± S.D.) | 3-min \(^{15}\text{N}\) PAD Digest Inter-peptide (mean ± S.D.) |
|-------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------------------|--------------------------------------------------|
| SIAQSIEK | \(5.33 \times 10^5\) | \(5.55 \times 10^5\) | \(4.84 \times 10^5\) | \(5.24 \times 10^5 ± 3.64 \times 10^4\) (6.95) | \(5.00 \times 10^5 ± 2.09 \times 10^4\) (4.18) |
| LGVILPVTK | \(4.83 \times 10^5\) | \(5.03 \times 10^5\) | \(4.86 \times 10^5\) | \(4.91 \times 10^5 ± 1.06 \times 10^4\) (2.18) |                                                   |
| LIYGIPQLIEYK | \(4.72 \times 10^5\) | \(5.20 \times 10^5\) | \(4.64 \times 10^5\) | \(4.85 \times 10^5 ± 3.05 \times 10^4\) (6.29) |                                                   |

### 2-h Digestion Protocol (\(5 \times 10^5\) CFU ml\(^{-1}\) spiked S. aureus)

|       | 2-h \(^{15}\text{N}\) PAD Digest 1 | 2-h \(^{15}\text{N}\) PAD Digest 2 | 2-h \(^{15}\text{N}\) PAD Digest 3 | 2-h \(^{15}\text{N}\) PAD Digest Intra-peptide (mean ± S.D.) | 2-h \(^{15}\text{N}\) PAD Digest Inter-peptide (mean ± S.D.) |
|-------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------------------|--------------------------------------------------|
| SIAQSIEK | \(4.74 \times 10^5\) | \(4.19 \times 10^5\) | \(4.73 \times 10^5\) | \(4.56 \times 10^5 ± 3.13 \times 10^4\) (6.86) | \(4.74 \times 10^5 ± 1.82 \times 10^4\) (3.83) |
| LGVILPVTK | \(4.96 \times 10^5\) | \(4.66 \times 10^5\) | \(5.14 \times 10^5\) | \(4.92 \times 10^5 ± 2.39 \times 10^4\) (4.85) |                                                   |
| LIYGIPQLIEYK | \(4.80 \times 10^5\) | \(4.57 \times 10^5\) | \(4.90 \times 10^5\) | \(4.76 \times 10^5 ± 1.69 \times 10^4\) (3.56) |                                                   |

### Overnight Digestion Protocol (\(5 \times 10^5\) CFU ml\(^{-1}\) spiked S. aureus)

|       | Overnight \(^{15}\text{N}\) PAD Digest 1 | Overnight \(^{15}\text{N}\) PAD Digest 2 | Overnight \(^{15}\text{N}\) PAD Digest 3 | Overnight \(^{15}\text{N}\) PAD Digest Intra-peptide (mean ± S.D.) | Overnight \(^{15}\text{N}\) PAD Digest Inter-peptide (mean ± S.D.) |
|-------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------------------|--------------------------------------------------|
| SIAQSIEK | \(5.42 \times 10^5\) | \(6.47 \times 10^5\) | \(5.60 \times 10^5\) | \(5.83 \times 10^5 ± 5.64 \times 10^4\) (9.67) | \(5.67 \times 10^5 ± 1.48 \times 10^4\) (2.61) |
| LGVILPVTK | \(5.06 \times 10^5\) | \(6.38 \times 10^5\) | \(5.53 \times 10^5\) | \(5.66 \times 10^5 ± 6.70 \times 10^4\) (11.84) |                                                   |
| LIYGIPQLIEYK | \(5.04 \times 10^5\) | \(6.30 \times 10^5\) | \(5.26 \times 10^5\) | \(5.54 \times 10^5 ± 6.87 \times 10^4\) (12.17) |                                                   |
reus quantitation and offering time-saving advantages over standard PAD methodologies. Traditional digestion protocols often include reduction and alkylation steps followed by lengthy trypsin incubation times that range from several hours to overnight. The rapid 3-min digest produced maximum peptide yields that showed no significant difference in peptide recoveries when compared with traditional digest preparations, thereby enhancing the rapidity of the method. Phage-amplified digest samples were quantified by LC-MS/MS configured for alternating column regeneration to further increase sample throughput. Using two columns, two pumps, and one 2-position 10-port valve allowed switching between columns for short cycle times from injection to injection. Extracted ion chromatograms of the labeled and unlabeled peptide isoforms showed co-elution of the peptide pairs with high retention time reproducibility and allowed the 15N signals to be used as retention time indicators for the native peptide signals, thus improving precision for quantifying peptide abundances. MRM MS allowed simultaneous quantitation of peptides from the phage capsid protein as a measure of S. aureus concentration and method specificity was enhanced by monitoring three ion transitional pairs for each peptide for a total of 12 independent ion transitions used for quantitation and confirmation of the phage protein of interest.

Although we have demonstrated the feasibility of this approach using cultivated S. aureus, Staphylococcal bacteriophage 53 and LC-MS/MS, this quantitative technique should be broadly applicable to other bacteria. The presented data encourages the continued development and use of isotopically labeled PAD MS-based methods for rapid bacterial quantification.

Although in recent years PAD has begun to emerge as an alternative to conventional assays for detection of bacterial pathogens, the technology is still in its infancy. The most prominent feature of PAD is the ability of the phage to amplify only in its bacterial target, allowing indirect detection with a high degree of specificity. The introduction of a combinatorial metabolic labeling PAD MS approach advances the field of PAD by providing quantitative results with short detection times, enhanced specificity, and improved sensitivity. Experimental design for quantitation of bacteria based on phage amplification used S. aureus strain 27694 and its corresponding bacteriophage. This strain was chosen solely as a representative test model and host range investigational studies would be necessary before diagnostic applications could be determined in a clinical context. There are many possible applications to the 15N PAD MS quantification method. The method uses the specificity of the bacteriophage to identify the bacteria of interest and should be able to accurately quantify target bacteria in co-infected samples and complex mixtures. Traditional techniques require an enrichment culture followed by plating and subsequent culturing of single colonies to obtain pure cultures. The 15N PAD MS method allows direct detection and accurate quantification of a target bacteria in the enrichment cultures. Further, the low limit of detection (≤5.0 × 10^4 CFU ml^-1) warrants investigation of analysis directly from some clinical and environmental samples. Because phage only amplify in viable bacterial cells, the use of bacteriophage as tools for drug, antimicrobial, and antibiotic susceptibility testing has been successfully demonstrated and testing the effectiveness of antibiotics on a S. aureus culture or clinical isolate is feasibly quantifiable with 15N PAD LC-MS/MS.

Bridging the gap between high complexity, low volume testing for research MS-based applications with less complex, rapid turnaround, and automated routine MS-based applications can be challenging. This method's current level of complexity makes it more compatible for complementing or confirming routine clinical diagnostics in higher end reference or specialized laboratories. However, isotopically labeled PAD coupled with MS possesses unique characteristics that are well-matched to be incorporated with ease into the workflow of microbiological laboratories and wider adoption of the technology is warranted. Mass spectrometers are becoming more common in clinical laboratories and all indications suggest that this trend will continue. Because of the simplicity of phage cultivation preparation and the low cost of required reagents, incorporating PAD into a user-friendly platform that offers cost-effective analysis is a practical outlook. Additionally, PAD’s ability to quantify live, viable bacteria in a variety of sample matrices without the need for confirmatory analyses makes it an attractive alternative when compared with PCR and many rapid culture methods. LC-MS/MS instruments are becoming increasingly simple to operate, have improved sensitivity and selectivity than many traditional bioanalytical assays, and offer universal detection and multiplexing capabilities that support timely analyses and a cost-effective investment.

As PAD matures and MS continues its emergence in clinical settings, this technique for detection and quantification of living pathogens should prove to be a powerful diagnostic tool for existing and emerging infectious diseases.

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23. Cagney, G., and Emili, A. (2002) De novo peptide sequencing and quantification of N-linked glycoproteins using hydrazide chemistry, stable-isotope-labeled internal standards. J. Chromatogr. A 949, 173–184
24. Durell, S. P., Rist, B., Gerber, S. A., Turecek, F., Gelb, M. H., and Aebersold, R. (2010) Quantitative proteomics by metabolic labeling of model organisms. Mol. Cell Proteomics 9, 11-24
25. Chakraborty, A., and Regnier, F. E. (2002) Global internal standard technology for comparative proteomics. J. Chromatogr. A 949, 173–184
26. Kristjansdottir, K., and Kron, S. J. (2010) Stable-isotope labeling for protein quantitation by mass spectrometry. Anal. Chem. 82, 7174-7185
27. Fridman, A., and Aebersold, R. (2007) Quantitative Proteomics by Stable Isotope Labeling and Mass Spectrometry In Methods in Molecular Biology, Vol. 367, pp. 209–218, Matthesies, R., Totowa, NJ
28. Goyla, V., and Aebersold, R. (2007) Quantitative Proteomics by metabolic labeling with stable isotopes. Drug Discovery Today: TARGETS 3, 11-15
29. Gouw, J. W., Kriegs, V., and Aebersold, R. (2010) Quantitative proteomics by metabolic labeling of model organisms. Mol. Cell Proteomics 9, 11-24
30. Walther, T. C., and Mann, M. (2010) Mass spectrometry-based proteomics in cell biology. J. Cell Biol. 190, 491–500
31. Nelson, C. J., Huttilin, E. L., Hegeman, A. M., Harms, A. C., and Sussman, M. R. (2007) Implications of 15N-metabolic labeling for automated peptide identification in Arabidopsis thaliana. Proteomics 7, 1279–1292
32. Schofield, D. A., Molineux, I. J., and Westwater, C. (2009) Diagnostic biomolecular assay for the detection of Yersinia pestis. J. Clin. Microbiol. 47, 3937–3948
33. Schababi, A., Zourobi, M., Allain, B., Marquette, C. A., Lawrence, M. F., and Mandeville, R. (2008) Bacteriophage-modified microarrays for the direct impediment detection of bacteria. Anal. Chem. 80, 9475-9482
34. Ripp, S., Jegier, P., Johnson, C. M., Brigat, J. R., and Sayler, G. S. (2008) Bacteriophage-activated bioluminescent sensing of Escherichia coli O157:H7. Anal. Bioanal. Chem. 391, 507–514
35. Madonna, A. J., Van Cuyk, S., and Voorhees, K. (2003) Detection of Escherichia coli using immunomagnetic separation and bacteriophage amplification coupled with magnetic-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 17, 257–263
36. Rees, J. C., and Voorhees, K. J. (2005) Simultaneous detection of two bacterial pathogens using bacteriophage amplification coupled with magnetic-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 17, 257–263
37. Reimann, R. W., Atchley, D. H., and Voorhees, K. J. (2007) Indirect detection of Bacillus anthracis using real-time PCR to detect amplified gamma phage DNA. J. Microbiol. Methods 68, 651–653
38. Goodridge, L., Chen, J., and Griffiths, M. (1999) Development and characterization of a fluorescent-bacteriophage assay for detection of Escherichia coli O157:H7. Appl. Environ. Microbiol. 65, 1397–1404
39. Pierce, C., Rees, J. C., and Barr, J. R. (2011) Detection of Staphylococcus aureus Using 15N-labeled bacteriophage amplification coupled with magnetic-assisted laser desorption/ionization-time-of-flight mass spectrometry. Anal. Chem. 83, 2286–2293
40. Adams, M. (1995) Bacteriophages, Interscience Publishers, NY
41. Keller, A., Nesvizhskii, A. I., Koler, E., and Aebersold, R. (2002) Empirical statistical model to estimate the accuracy of peptide identifications made...
by MS/MS and database search. Anal. Chem. 74, 5383–5392
47. Nesvizhskii, A. I., Keller, A., Kolker, E., and Aebersold, R. (2003) A statistical
model for identifying proteins by tandem mass spectrometry. Anal.
Chem. 75, 4646–4658
48. Turapov, O. A., Mukamolova, G. V., Bottrill, A. R., and Pangburn, M. K.
(2008) Digestion of native proteins for proteomics using a thermocycler.
Anal. Chem. 80, 6093–6099
49. Moura, H., Terilli, R., Woolfitt A., Gallegos-Candela, M., McWilliams, L.,
Solano, M., Pirkle, J., and Barr, J. R. (2011) Studies on botulinum
neurotoxins Type/C1 and mosaic/DC using Endopep-MS and proteom-
ics. FEMS Immunol. & Medical Microbiol. 61, 288–300
50. Yu, Y. Q., Gilar, M., Lee, P. J., Bouvier, E. S., and Gebler, J. C. (2003)
Enzyme-friendly, mass spectrometry-compatible surfactant for in-solu-
tion enzymatic digestion of proteins. Anal. Chem. 75, 6023–6028
51. Suder, P., Bierczynska, A., König, S., and Silberring, J. (2004) Acid-labile
surfactant assists in-solution digestion of proteins resistant to enzymatic
attack. Rapid Commun. Mass Spectrom. 18, 822–824
52. Mo, W., Ma, Y., Takao, T., and Neubert, T. A. (2000) Sequencing of oxidized
methionine-containing peptides for protein identification. Rapid Com-
mun. Mass Spectrom. 14, 2080–2081
53. Taylor, S. W., Fahy, E., Murray, J., Capaldi, R. A., and Ghosh, S. S. (2003)
Oxidative post-translational modification of tryptophan residues in car-
diac mitochondrial proteins. J. Biol. Chem. 278, 19587–19590
54. Atrih, A., Turnock, D., Sellar, G., Thompson, A., Feuerstein, G., Ferguson,
M. A., and Huang, J. T. (2010) Stoichiometric quantification of Akt phos-
phorylation using LC-MS/MS. J. Proteome Res. 9, 743–751
55. Payne, R. J., and Jansen, V. A. (2001) Understanding bacteriophage ther-
apy as a density-dependent kinetic process. J. Theor. Biol. 208, 37–48
56. Kasman, L. M., Kasman, A., Westwater, C., Dolan, J., Schmidt, M. G., and
Norris, J. S. (2002) Overcoming the phage replication threshold: a math-
ematical model with implications for phage therapy. J. Virol. 76,
5557–5564
57. Sutton, S. (2011) Counting Colonies, In The Microbiology Network, Ac-
cessed online February 17, 2011. http://www.microbiol.org/resources/
monographwhite-papers/counting-colonies
58. Norrgran, J., Williams, T. L., Woolfitt, A. R., Solano, M. I., Pirkle, J. L., and
Barr, J. R. (2009) Optimization of digestion parameters for protein quan-
tification. Anal. Biochem. 393, 48–55

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