



Important techniques in today's biomedical science research that PhD candidates should be exposed to: a perspective from the FASEB journal

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Summary: The need for best evidence has driven researchers into multidisciplinary, collaborative approaches which have become mainstay in today's biomedical science. The multidisciplinary and collaborative approaches to research in research-intensive academic medical centres in the USA and in other countries of affluence has brought in significant advancement in knowledge as well as colossal progress and financial benefits. Therefore the author speculates that for Nigerian and other African PhD graduates in the basic medical sciences to become successful researchers, effective peer reviewers, reliable mentors, and progressive administrators in research-based academia, they need some exposure to multidisciplinary approaches to research during their subject-based training. The present report sought to substantiate this need. Thirty three published articles in the April 2012 FASEB Journal were studied for the methodologies employed and the results showed that the papers utilized an average of nine major biomedical science techniques, 9 being the mean, median, and mode showing the global *status quo* of diversity of methodology per scientific paper. The most popular procedures and techniques recorded in more than 1/3 of the published articles were: cell isolation; cell culture; *in vivo* or *in situ* whole animal studies; animal models of disease; gene/protein expression, sequencing and cloning; transfection, constructs, and genomic interference and silencing; western blotting; fluorescence and confocal microscopy; ELISAs and cell-based assays; and ready-made biotech assay kits. The most popular statistics testing were various forms of student's t-tests at 0.05 confidence levels and ANOVAs. The GraphPad Prism software was the most frequently used statistic software.

Keywords: Techniques, Biomedical Science, PhD, Research

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Manuscript Accepted: January, 2013

INTRODUCTION

The Doctor of Philosophy program is traditionally aimed at producing subject experts who would be teachers, researchers, and consultants in their field. In the basic medical sciences, traditional subjects of PhD's include anatomy, physiology, biochemistry, microbiology, pathology, and pharmacology. As our body of knowledge expands, the need for more subject areas for PhD's has also increased and we have PhD graduates in specialized areas such as cell biology and molecular biology or in the broader biomedical science. The subject-based character of PhD basic science and biomedical science programs has undergone change in some research intensive academic medical centres. In Nigeria, and perhaps in other African countries, medical schools rely mainly on government and school fees for their funding. In research intensive institutions in the USA, medical schools rely on research grants (mainly from the government) and clinical earnings for their funding

(Bloom 1992). In such institutions, changes have been made to ensure that faculty in any medical school department are a multidisciplinary group capable of wielding the research capability that satisfies grant awarders (Handricson *et al.*, 1993; Reynolds *et al.*, 1995, John, 2003). Hence it is possible to have situations whereby in subject departments, while teaching is largely faithful to the subject turf, research is absolutely promiscuous. The need for best evidence has driven researchers into multidisciplinary, collaborative approaches which have become mainstay in today's biomedical science (John, 2003). Therefore, while PhD candidates learn the theory of their subject areas, they also need to acquire the skills and methodologies of their subject areas plus some skills and methodologies across the broader biomedical science comprised of all the basic sciences. The multidisciplinary and collaborative approaches to research in research intensive academic medical centres in the USA and in other countries of affluence has brought in significant advancement in

knowledge as well as colossal progress and financial benefits. In Nigeria (and perhaps many parts of Africa), state-of-the-arts facilities are needed to support profitable research (John 2010). Technical staff in some Nigerian (and perhaps other African) institutions may also need an upgrade of their skills to be able to service profitable collaborative, multidisciplinary research (John, 2009, 2011a). This is because lucrative research is generally funded by interested stake-holders who need to ensure desired results and experience shows that best evidence comes from state-of-the-arts facilities and collaborative, multidisciplinary efforts. Therefore the author speculates that for PhD graduates in the basic medical sciences to become successful researchers, effective peer reviewers, reliable mentors, and progressive administrators in research-based academia, they need some exposure to multidisciplinary approaches to research during their subject-based training. The present report actualizes this as a fact. Thirty three published articles in the April 2012 FASEB journal were studied for the methodologies employed and the results showed that the papers utilized an average of nine major biomedical science techniques, 9 being the mean, median, and mode showing the global *status quo* of diversity of methodology per scientific paper.

MATERIALS AND METHODS

The author here presents a study of thirty three peer-reviewed scientific publications in the April 2012 issue of the FASEB Journal [Volume 26(4)]. The FASEB Journal was chosen because it is published by the Federation of American Societies for Experimental Biology (FASEB). It is a time-tested federation established in 1912 by three societies and celebrating its centenary in 2012. From the FASEB website: "FASEB's members are scientific societies that share a common vision for the advancement of research and education in biological and biomedical sciences. They are listed below in ascending chronological order.

- The American Physiological Society (APS)
- American Society for Biochemistry and Molecular Biology (ASBMB)
- American Society for Pharmacology and Experimental Therapeutics (ASPET)
- American Society for Investigative Pathology (ASIP)
- American Society for Nutrition (ASN)
- The American Association of Immunologists (AAI)
- American Association of Anatomists (AAA)
- The Protein Society (PS)
- Society for Developmental Biology (SDB)
- American Peptide Society (APEPS)
- Association of Biomolecular Resource Facilities (ABRF)

- The American Society for Bone and Mineral Research (ASBMR)
- The American Society for Clinical Investigation (ASCI)
- Society for the Study of Reproduction (SSR)
- The Teratology Society (TS)
- The Endocrine Society (TES)
- The American Society of Human Genetics (ASHG)
- Environmental Mutagen Society (EMS)
- International Society for Computational Biology (ISCB)
- American College of Sports Medicine (ACSM)
- Biomedical Engineering Society (BMES)
- Genetics Society of America (GSA)
- American Federation for Medical Research (AFMR)
- The Histochemical Society (HCS)
- Society for Pediatric Research (SPR)
- Society for Glycobiology (SFG)

The FASEB journal is published monthly. From the BIOXBIO website information

(<http://www.bioxbio.com/if/html/FASEB-J.html>) the recent impact factors and cites of the FASEB journal articles are in the Table below.

Table 1. Recent Impact Factors of the FASEB Journal

Year	Impact Factor (IF)	Total Articles	Total Cites
2011	5.712	409	38304
2010	6.515	462	38538
2009	6.401	410	35849
2008	7.049	412	34300

The FASEB Journal is described as "The Journal That Covers All The Life Sciences And The Life of Science" (according to the slogan on the inside front cover of the issue studied). Perhaps the findings of this study will give more life to Nigerian (indeed, African) biomedical science.

Each of the 33 published research articles was studied for major techniques utilized as presented in Tables 2-4. The techniques examined include the biological resources utilized. The results show the number of articles reporting particular major techniques presented as sums and used to determine the popularity of individual techniques

RESULTS

Table 2 shows the biomedical science procedures and biological resources utilized by the 33 research articles studied. Biological resources were mainly from animals and cell culture. Half of the articles specifically mentioned ethical review approval (17/33). The most popular biological resource was cell culture (23/33). The results show that the use of artificially modified biological resources was popular; 19/33 used some form of transfection, constructs, or genomic interference and silencing. Of the 33 articles, 6 used human subjects, 3 used human

patients, 1 used human parts, 17 used *in vivo* or *in situ* whole animal studies out of which 13 used animal models of disease. Out of those who reported animal studies, 10 used transgenic animals and 4 used isolated organ or *in vitro* preparations. More than 1/3 researchers isolated cells for their studies (13/33), while 2 articles reported use of biopsies, and 12 articles reported use of tissue slices or sections and 7 reported use of tissue homogenates or tissue extracts. Tables 3 and 4 show the major techniques utilized in experimental procedures reported by the 33 articles. Figure 1 depicts the order of popularity of techniques utilized. Amongst the most popular, the numbers recorded per technique were: gene/protein expression, sequencing and cloning (28/33); western blotting (19/33); fluorescence or confocal microscopy (15/33); ELISAs and cell-based assays (14/33); specific mention of use of biotech assay kits (11/33). These techniques were recorded by at least 1/3 of the published articles.

Table 5 shows the total numbers of major techniques utilized per article. These numbers ranged

from 5 to 13; the average is 9.06; the mode is 9; and the median is 9.

Table 6 shows the general statistical methods recorded by the 33 research articles. More than 1/3 of the papers used one form of student's t-test (23/33), one form of ANOVA (19/33), one form of post-hoc tests (12/33), and the 0.05 confidence level (21/33). The majority (10/33) reported the use of GraphPad Prism as their statistics package. Other statistical procedures or packages were rare: Mirakaj *et al.* used normal distribution and Bonferroni multiple comparison; Rodger *et al.* used normal distribution with ANOVA and a posthoc test; Tal *et al.* used Pearson's correlation; Wang *et al.* used Chi squared; Huttemann *et al.* used SPSS package; Zuo *et al.*, used JMP9; Khapersky *et al.* used MS Excel; Hifumi *et al.* and Engel *et al.* used StatView. Most of the statistics was done at 5% confidence levels and p recorded as <0.05. Other confidence levels were rare: Tal *et al.* and Hwang *et al.* used 0.01; Hwang *et al.* also used 0.001; and Hifumi *et al.* used 0.02.

Table 2. Biomedical science procedures and biological resources utilized by the 33 research articles in the April 2012 issue of the FASEB Journal

AUTHORS	ethical review approval	human subjects	human patients	human parts	whole animals, in vivo, in situ	animal disease models	transgenic animals	in vitro preparations, isolated organs	cell isolation	cell culture	transfection, constructs, interference, silencing	human biopsies	tissue slices, sections	tissue homogenates, tissue extracts
Huttemann <i>et al.</i> ,	*				*		*	*					*	*
Pacios <i>et al.</i> ,					*	*							*	
Levette <i>et al.</i> ,	*	*										*		*
Zhang <i>et al.</i> ,										*	*			
Tal <i>et al.</i> ,	*				*						*			*
Kemaladewi <i>et al.</i> ,								*	*	*	*			
Durgadoss <i>et al.</i> ,	*	*			*			*	*	*				*
Hwang <i>et al.</i> ,									*	*	*			
Zuo <i>et al.</i> ,					*		*	*	*	*	*			
Wang <i>et al.</i> ,	*				*	*		*	*	*			*	*
Dancyger <i>et al.</i> ,											*			
Foller <i>et al.</i> ,			*							*	*			
Ramer <i>et al.</i> ,	*		*		*	*		*	*	*	*		*	
Mirakaj <i>et al.</i> ,	*	*			*	*	*	*	*	*	*		*	*
Yamashita <i>et al.</i> ,					*	*	*	*	*	*			*	
Mijouin <i>et al.</i> ,									*	*	*			
Tarcic <i>et al.</i> ,									*	*	*			
Rodger <i>et al.</i> ,	*				*	*	*						*	
Hifumi <i>et al.</i> ,	*	*				*		*	*	*	*			
Engel <i>et al.</i> ,	*				*	*		*	*	*	*		*	
Khapersky									*	*	*			
Bosmann <i>et al.</i> ,	*				*	*	*	*	*	*	*			
Liu <i>et al.</i> ,	*				*	*	*				*		*	
Wann <i>et al.</i> ,					*	*	*	*	*	*	*			
Woo <i>et al.</i> ,				*	*	*	*	*	*	*	*			
Goudet <i>et al.</i> ,	*					*		*	*	*	*		*	
Begum <i>et al.</i> ,	*				*								*	*
Hirota <i>et al.</i> ,	*	*	*						*	*	*	*		
Barison <i>et al.</i> ,									*	*	*			
Lam <i>et al.</i> ,	*				*	*		*						
Chan <i>et al.</i> ,									*	*	*			
Singh <i>et al.</i> ,	*				*	*	*	*					*	
Serhan <i>et al.</i> ,		*				*			*	*	*			*

Table 3. Some key biomedical science techniques utilized by the 33 research articles in the April 2012 issue of the FASEB Journal

AUTHORS	ELISAs, cell-based assays	immunoprecipitation, pull-down, CHIP, hybridization	electrophoresis	western blotting	immunohistochemistry, FISH	light microscopy, digital microscopy	fluorescence, confocal or laser scanning microscopy	flow cytometry, FACS	electron microscopy	electrophysiology, patch clamp	gene expression, sequencing, cloning, genotyping, PCR	FISH	spectrophotometry	liquid chromatography, mass spectrometry
Huttemann <i>et al.</i> ,				*					*		*			
Pacios <i>et al.</i> ,					*	*	*				*			
Levette <i>et al.</i> ,									*				*	
Zhang <i>et al.</i> ,	*			*			*						*	
Tal <i>et al.</i> ,											*			
Kemaladewi <i>et al.</i> ,				*	*						*			
Durgadoss <i>et al.</i> ,	*			*							*			
Hwang <i>et al.</i> ,	*			*							*			
Zuo <i>et al.</i> ,	*			*			*	*			*			
Wang <i>et al.</i> ,											*		*	*
Dancyger <i>et al.</i> ,			*	*							*			
Foller <i>et al.</i> ,				*			*	*			*			
Ramer <i>et al.</i> ,	*			*							*			
Mirakaj <i>et al.</i> ,	*			*			*	*			*			
Yamashita <i>et al.</i> ,						*	*	*			*	*		
Mijouin <i>et al.</i> ,	*	*		*			*				*			
Tarcic <i>et al.</i> ,	*	*	*	*			*				*	*		
Rodger <i>et al.</i> ,	*						*			*				
Hifumi <i>et al.</i> ,				*			*				*			
Engel <i>et al.</i> ,	*			*	*		*			*				
Khaperskyy		*		*			*				*			
Bosmann <i>et al.</i> ,	*					*	*	*			*			
Liu <i>et al.</i> ,									*		*			
Wann <i>et al.</i> ,				*			*				*			
Woo <i>et al.</i> ,		*		*	*		*				*			
Goudet <i>et al.</i> ,	*									*				
Begum <i>et al.</i> ,		*									*			
Hirota <i>et al.</i> ,	*			*	*			*			*			
Barison <i>et al.</i> ,	*	*							*		*			*
Lam <i>et al.</i> ,											*			
Chan <i>et al.</i> ,				*				*			*			
Singh <i>et al.</i> ,					*					*	*			
Serhan <i>et al.</i> ,										*				*

DISCUSSION

The PhD graduate in present day biomedical science will end up in several different roles including: teaching; research; lab demonstrations; peer review of research articles before publication; review of grant applications; steering of scientific discussions, workshops, conferences, and other forums; consultancy; and academic administration. Most of these duties are research-related. While disciplinary theory is sufficient for effectively teaching in the subject area, it is far from sufficient for state-of-the-arts research and research-based duties that the PhD candidate may eventually become involved in. The

present report shows the state-of-the-arts in biomedical science research publications. Each paper published in the April 2012 issue of the FASEB Journal uses an average of 9 major techniques borrowed from varied disciplines. The department(s) of origin, the institution(s) of origin, and the authors of the peer reviewed papers do not make a difference to this fact. The mean, mode, and median number of major biomedical science techniques utilized by the 33 articles is 9. PhD candidates should therefore bear in mind that to publish a good scientific research paper in a high impact journal, they need to include several different techniques and approaches that congruently prove the same hypothesis.

Table 4. More key biomedical science techniques utilized by the 33 research articles in the April 2012 issue of the FASEB Journal

AUTHORS	microplate readers, multilabel counters, optical imaging	biotech kits	microarray	video recording	computed tomography (CT)	MRI	mass spectrophotometry	crystallization, structure determination, SPR	hybridization, southern blot	protein purification	bioinformatics, molecular modelling
Huttemann <i>et al.</i> ,											
Pacios <i>et al.</i> ,		*									
Levette <i>et al.</i> ,		*									
Zhang <i>et al.</i> ,	*	*									
Tal <i>et al.</i> ,		*	*	*	*						
Kemaladewi <i>et al.</i> ,	*										
Durgadoss <i>et al.</i> ,		*									
Hwang <i>et al.</i> ,	*	*									
Zuo <i>et al.</i> ,								*			
Wang <i>et al.</i> ,		*									
Dancyger <i>et al.</i> ,								*			
Foller <i>et al.</i> ,		*									
Ramer <i>et al.</i> ,											
Mirakaj <i>et al.</i> ,											
Yamashita <i>et al.</i> ,		*									
Mijouin <i>et al.</i> ,								*		*	
Tarcic <i>et al.</i> ,			*								
Rodger <i>et al.</i> ,				*	*						
Hifumi <i>et al.</i> ,	*							*			*
Engel <i>et al.</i> ,											
Khapersky											
Bosmann <i>et al.</i> ,											
Liu <i>et al.</i> ,						*	*				
Wann <i>et al.</i> ,		*									
Woo <i>et al.</i> ,											
Goudet <i>et al.</i> ,								*			
Begum <i>et al.</i> ,						*					*
Hirota <i>et al.</i> ,											
Barison <i>et al.</i> ,								*			
Lam <i>et al.</i> ,											
Chan <i>et al.</i> ,									*		
Singh <i>et al.</i> ,		*									
Serhan <i>et al.</i> ,	*										

The actual techniques utilized to test any hypothesis will depend on the hypothesis being tested but the present report indicates that there are some procedures and techniques that have a high frequency of usage. These “universal techniques” utilized by at least 1/3 (11/33) of the published research articles examined include: ethical review approval (17/33);

cell culture (23/33); cell isolation (13/33); transfection, constructs, or genomic interference and silencing (19/33); gene/protein expression, sequencing and cloning (28/33); *in vivo* or *in situ* whole animal studies (17/33); animal models of disease (13/33); western blotting (19/33); fluorescence or confocal microscopy (15/33); ELISAs and cell-based assays

(14/33); ready-made biotech assay kits (11/33). For statistical analysis, more than 1/3 of the articles used: various forms of student's t-tests at $p < 0.05$, various forms of ANOVAs, various posthoc tests, and the GraphPad Prism software. The popularity of these techniques is based on their utility and the results that can be derived from them, irrespective of whether they are easy or difficult; laborious or quick; inexpensive or expensive, convenient or cumbersome. They are used across the basic science disciplines and PhD candidates from any discipline should be familiar with them. This is because these PhDs would need to know and utilize

these techniques in order to produce scientific papers that can be accepted in high impact journals; to write competitive research grant applications to obtain funding for their own research; to be effective in reviewing the scientific papers of up-to-date peers that utilize multidisciplinary techniques; to be effective in steering scientific conferences, seminars, technical forums and other professional activities and to manage their proceedings; to be competent in directing research administration and in providing research infrastructure; to be reliable mentors of future scientists; and to be leaders in their chosen field of research

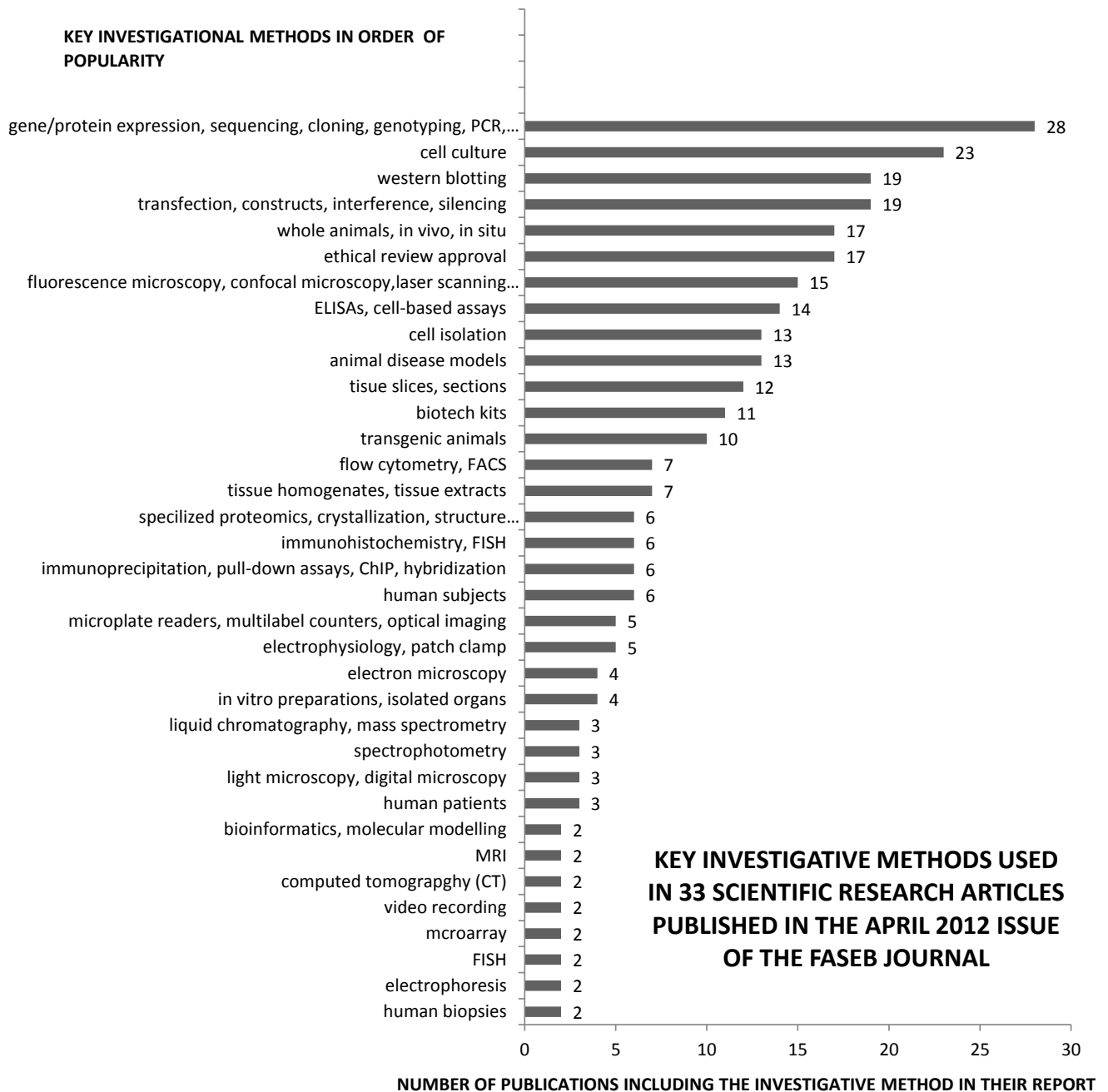


Figure 1. Key investigational methods in order of popularity as utilized in the 33 research articles published in the April 2012 issue of the FASEB Journal

Table 5. Number of key biomedical science techniques utilized by each of the 33 research articles in the April 2012 issue of the FASEB Journal

AUTHORS	TOTAL NUMBER OF KEY METHODS UTILIZED PER RESEARCH ARTICLE
Huttemann et al.,	9
Pacios et al.,	8
Levette et al.,	8
Zhang et al.,	8
Tal et al.,	9
Kemaladewi et al.,	7
Durgadoss et al.,	10
Hwang et al.,	7
Zuo et al.,	11
Wang et al.,	12
Dancyger et al.,	5
Foller et al.,	8
Ramer et al.,	11
Mirakaj et al.,	13
Yamashita et al.,	12
Mijouin et al.,	9
Tarcic et al.,	10
Rodger et al.,	10
Hifumi et al.,	12
Engel et al.,	11
Khaperskyy	6
Bosmann et al.,	11
Liu et al.,	9
Wann et al.,	10
Woo et al.,	11
Goudet et al.,	8
Begum et al.,	8
Hirota et al.,	10
Barison et al.,	7
Lam et al.,	5
Chan et al.,	6
Singh et al.,	9
Serhan et al.,	9

Infrastructure in Nigerian and other African universities need to be brought up-to-date (John 2009, 2010) to facilitate that PhD candidates' gain research capacity in preparation for their necessary future roles in the academic and other spheres.

As time goes on, it will be necessary to repeat evaluation of important techniques necessary for young biomedical scientists to know. There are always driving forces of change (John 2011b). New

Table 6. Key statistical methods and software utilized in the 33 research articles published in the April 2012 issue of the FASEB Journal

AUTHORS	student's t tests	ANOVAs	post hoc	p<0.05	Prism/GraphPad
Huttemann <i>et al.</i>		*	*	*	
Pacios <i>et al.</i>		*		*	
Levette <i>et al.</i>	*			*	
Zhang <i>et al.</i>	*			*	
Tal <i>et al.</i>	*	*	*	*	*
Kemaladewi <i>et al.</i>	*			*	
Durgadoss <i>et al.</i>	*	*	*	*	
Hwang <i>et al.</i>	*	*		*	*
Zuo <i>et al.</i>	*	*	*	*	
Wang <i>et al.</i>	*	*		*	*
Dancyger <i>et al.</i>	*	*			*
Foller <i>et al.</i>	*			*	
Ramer <i>et al.</i>	*	*	*	*	*
Mirakaj <i>et al.</i>	*	*		*	
Yamashita <i>et al.</i>	*		*	*	
Mijouin <i>et al.</i>					
Tarcic <i>et al.</i>		*	*		
Rodger <i>et al.</i>		*	*		
Hifumi <i>et al.</i>	*				
Engel <i>et al.</i>	*	*	*	*	
Khaperskyy <i>et al.</i>		*			
Bosmann <i>et al.</i>	*	*			*
Liu <i>et al.</i>					*
Wann <i>et al.</i>	*	*			
Woo <i>et al.</i>	*	*	*	*	*
Goudet <i>et al.</i>	*			*	
Begum <i>et al.</i>		*	*	*	*
Hirota <i>et al.</i>	*	*	*	*	*
Barison <i>et al.</i>					
Lam <i>et al.</i>	*			*	
Chan <i>et al.</i>					
Singh <i>et al.</i>	*			*	
Serhan <i>et al.</i>	*	*	*	*	
TOTAL	23	19	12	21	10

techniques may come, old ones may become obsolete, but all the time, scientists, especially in developing institutions, need to try to keep up-to-date in order to do successful and rewarding research. Direction of education has been the focus of very few studies in medical schools. Hendricson *et al.*, 1998; Reynolds *et al.*, 1995; Bryan 1994; and Huppatz, 1996 – were focussed on medical education turning out doctors. John (2003) was focussed on basic science education turning out basic medical scientists. Such studies help to ensure that educational outcomes are rewarding to the investors (government or private).

PhD candidates in the basic medical sciences who look forward to successful careers in biomedical science as global experts should be conversant with and be able to utilize at least 9 popular biomedical science techniques which include: cell isolation; cell culture; *in vivo* or *in situ* whole animal studies;

animal models of disease; gene/protein expression, sequencing and cloning; transfection, constructs, and genomic interference and silencing; western blotting; fluorescence and confocal microscopy; ELISAs and cell-based assays; and ready-made biotech assay kits. For statistical testing, PhD candidates should be familiar with the various forms of student's t-tests at 0.05 confidence levels, ANOVAs, and the use of GraphPad Prism software as these appear to be the most popularly utilized.

Acknowledgement

Thanks to the FASEB for a donation of the April 2012 issue of the FASEB Journal, given at their exhibition stall at Experimental Biology 2012, San Diego, California.

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