LESSONS LEARNED ABOUT SPACEFLIGHT AND CELL BIOLOGY EXPERIMENTS

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Hughes-Fulford, M. Lessons learned about spaceflight and cell biology experiments. *J. Grav. Physiol. 11(1):105-110, 2004.* Conducting cell biology experiments in microgravity can be among the most technically challenging events in a biologist's life. Conflicting events of spaceflight include waiting to get manifested, delays in manifest schedules, training astronauts to not shake your cultures and to add reagents slowly, as shaking or quick injection can activate signaling cascades and give you erroneous results. It is important to select good hardware that is reliable. Possible conflicting environments in flight include g-force and vibration of launch, exposure of cells to microgravity for extended periods until hardware is turned on, changes in cabin gases and cosmic radiation. One should have an on-board 1-g control centrifuge in order to eliminate environmental differences. Other obstacles include getting your funding in a timely manner (it is not uncommon for two to three years to pass between notification of grant approval for funding and actually getting funded). That said, it is important to note that microgravity research is worthwhile since all terrestrial life evolved in a gravity field and secrets of biological function may only be answered by removing the constant of gravity. Finally, spaceflight experiments are rewarding and worth your effort and patience.

key words: Microgravity

INTRODUCTION

As an astronaut on STS-40 and principle investigator on 56, 76, 81, 84, 107 and an upcoming Space Station experiment, I have noted many common challenges and witnessed mistakes made by myself and/or others. With the return to flight in 2005, space biology experiments will once again enter weightlessness and we need to be prepared. I have several "lessons learned" that I would like to share with fellow astronauts, investigators and administrators.

First, consider the vibration and gravity forces that your experiments will be exposed to while in transport from the lab to the shuttle and during launch to orbit. As a crewmember on STS-40, my first thoughts during my launch and our ten minute ascent to orbit was "OhmyGosh what is this doing to our experiments? " Launch is definitely shake, rattle and roll time, so take care to fly your samples in a quiescent state, and if that is impossible, try to dampen forces by careful packing and hardware design and dampened storage areas.

When I returned to my laboratory after my mission, the first experiments were to go the AMES research center and put my osteoblasts on the human rated centrifuge for a launch G-force profile and to the vibration hardware testing area for a vibration launch profile. We found that vibration caused significant induction (Fig. 1) of genes like *c-fos, c-myc* and significant downregulation of *osteocalcin* and *TGFβ1* (1, 2). The gravity launch profile also demonstrated (Fig. 2) that *c-fos* was significantly upregulated and osteocalcin was significantly downregulated (1). With that knowledge, Marian Lewis and I conducted successful osteoblast experiments on STS-56 by flying quiescent osteoblasts going to orbit.

This brings up another point; for accurate results, you should collect the samples while in orbit. Many times on-orbit sample collection is difficult and you need to stand firm as the experimental parameters are set. If you want to test the effect of micro-

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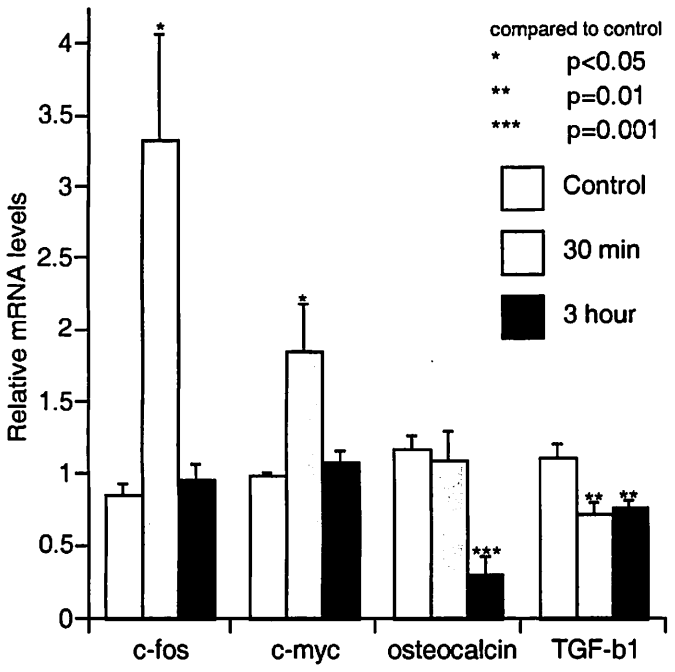

Figure 1. Alterations in mRNA levels in osteoblasts subjected to space shuttle launch vibration profile. All measurements were corrected to the internal standard cyclophilin for each data point ± SD. Used with permission *FASEB J.* 11:493-497, 1997.

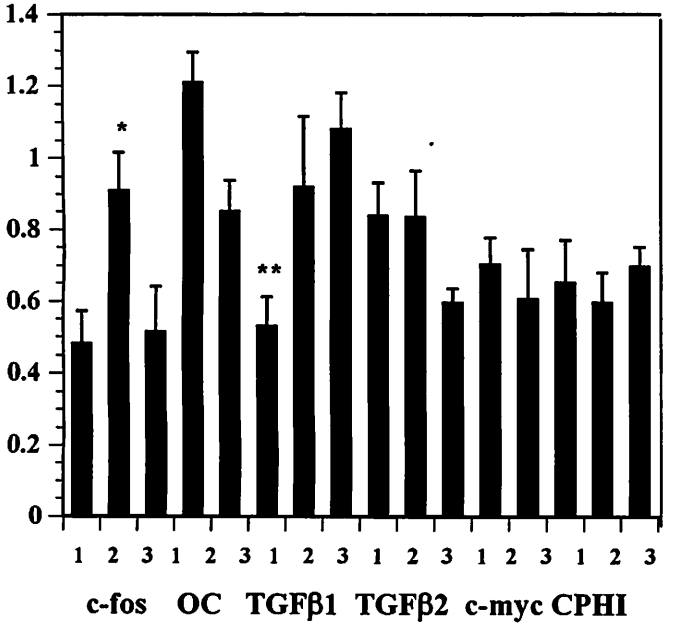

Figure 2. Summary of alterations in mRNA levels due to centrifugation. Quantitation of PCR band intensity for each gene and time transcription of the *c-fos* gene after centrifugation runs. Values shown were calculated by taking the ratio of the band intensity for each gene to the band intensity of the signal amplified from the same RT reaction. There are groups of three-. 1) samples not subject to centrifugation 2) cells fixed 30 min after centrifugation and 3) cells fixed after 3 hours. *, $P < 0.05$ (Student's t test) compared to mean of *c-fos*± SD. **, $P < 0.005$ compared to mean of OC control. Used with permission *Exp. Cell Res.* 228:168-171, 1996.

gravity, you must have activation and collection in orbit. I know of several people that did not, and they found that is hard to publish in a good journal without doing so. Many times investigators are told that is impossible to fix cultures in flight- this is a huge mistake since many hours will pass from the end of the experiment until de-orbit. The samples are exposed to the forces of re-entry, and another 4-8 hours pass after landing until the scientist receives the samples. All of this adds up to a host of conflicting environments that could easily cause erroneous results and inaccurate hypothesis. Were you results and conclusions a consequence of microgravity or a result of re-entry forces? You (and the world) may never know.

Another pitfall is flying an inadequate number of samples to get statistics from your experiment. Insist on n=4 or greater sample size per condition. Many people have published with an n=1 and were forced into low impact journals (and poor data gives NASA science a bad reputation). I ask you, would you attempt an experiment on the ground with only one sample and if so do think you are going to be published? I don't think so. If anything, spaceflight experiments need more rigor than ground based experiments for several reasons: first, you are most likely the first to do your experiment in microgravity and second, your results could be of utmost importance to understanding basic life science principals- so be exact and accurate make sure your results stand up to statistical analysis. So the lesson here is to have enough samples to get statistical results, accept no substitutes.

On STS-56 we found that the osteoblasts did not grow well in flight and had major alterations in the nuclear structure and cytoskeleton (Fig. 3) when in microgravity (3). After this experiment, I started thinking about normal gravity forces here on Earth. I realized at that we had really needed a low shear onboard 1g centrifuge to approximate normal Earth's gravity during the microgravity experiment. Using an onboard 1-g control would insure that we accounted for other pre-experiment flight variables such as cabin gases, vibration, launch forces, cosmic radiation and lack of diffusion in samples. Therefore when we got the chance to fly the osteoblast using the BIORACK on STS-76, 81 and 84, we asked for an onboard 1-g centrifuge and were lucky enough to get it.

On to proper storage of the samples that are fixed in microgravity. Ensure that you have appropriate storage of your samples, for instance, if you are storing media to analyze cytokines or eicosanoids (prostaglandins) and other labile molecules, make sure you freeze the samples in flight. There was one

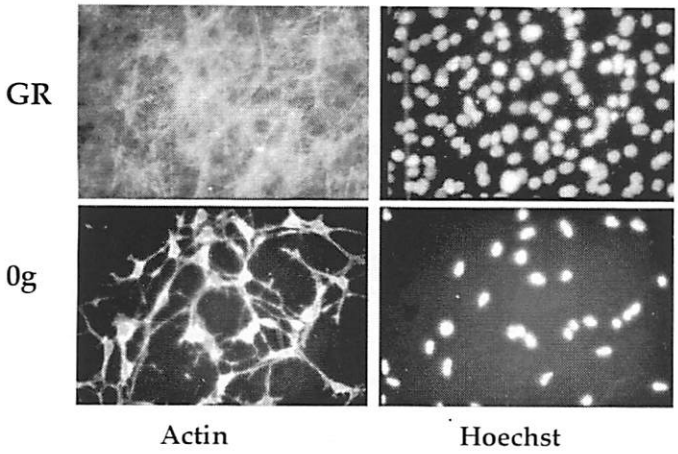

Figure 3. Changes in MC3T3-E1 cytoskeleton and elongation of nuclei in microgravity. Structure of the F-actin cytoskeleton of 0g and ground controls stained with rhodamine phalloidin visualized with a Zeiss Neofluar 100x oil objective. Nuclei were stained with Hoechst 33258. Photos were from STS-56 osteoblasts activated for four days in flight or in ground controls. Used with permission *Exp. Cell Res.* 224:103-109, 1996.

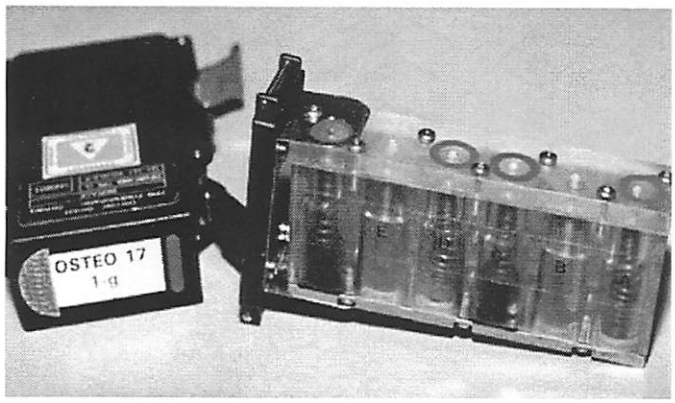

Figure 4. Plungerbox and type one container used on STS 76, 81 and 84 BioRack.

instance where media was stored in orbit for later prostaglandin analysis. Samples in flight were stored at room (cabin) temperature and saved until return. PGE_2 analysis showed very little prostaglandin content for the 10 days prior to landing, at which point the osteoblasts were once again able to make PGE_2- in reality, PGE_2 is labile at room temperature and breaks down, this is why inflight samples had less PGE_2 . Investigators were only able to see the compound in the freshest samples. Lesson learned: store ground samples under same conditions as flight samples and store the samples appropriately or you might come to the wrong conclusion.

Another potential pitfall is hardware flow environment, make sure that your hardware has no flow or shear forces. Studies have shown that shear forces and flow can stimulate signaling cascades and turn on genes without other factors present (4-9). Our recent studies on the ground have demonstrated that quiescent osteoblasts have a mechanical stress threshold for ERK activation (6), it is possible that this is one signal cascade that is not working in spaceflight and only experimentation in microgravity will answer the question. Regardless of you choice of cell, your hardware selection criteria must include elimination of constant flow or sudden injection of liquids that may induce gene expression. In addition, make sure your hardware will house ample samples to get all the analysis you desire. Another situation that has occurred repeatedly is that hardware is not delivered until less than six months prior to flight and the team does not have time to complete ground based experiments or acquire the experience to complete the job at hand. Moreover, without the hardware, it is hard to guess which solutions (preservatives or lysis buffers for example) are the best for flight. Lesson learned: get your hardware EARLY. Another tip about hardware - try to keep it simple. On the STS-76, 81 and 84 Biorack experiments, we had excellent luck with the hardware (Fig. 4) , but it was quite complicated and was a bear to clean and sterilize without autoclaving (the plastic cracks with autoclaving). We show a expansion diagram of the plunger box components, all had to be sterilized before assembly (Fig. 5).

Keep your experiments elegant and simple. The crew schedule is tight and an overly complicated experimental protocol that uses excess time can eliminate your experiment from consideration. A simple protocol and reliable hardware can streamline your science and can lead you to the road of success and understanding. Another heads up to the principal investigator, ask for all upcoming required paperwork ahead of time, sometimes the request comes in while you are on travel leaving you with little or no time to respond. Stay ahead of the game in the paperwork department and you will be much happier. NASA will ask for all the MSDSs for all your experimental supplies, so keep them handy and keep yourself happy.

Finally, and most importantly, start your full-up (hardware, personnel and supplies in place) experiment two years before flight. Get your money for personnel and supplies early. A word of caution, in all my experience, I never received my flight grant money in a timely manner. I was asked to evaluate hardware, hardware materials without personnel or adequate supply support. I was asked to set experiment parameters for flight without the opportunity to test them on the ground. This is also the experience of a majority of other scientists I have compared notes with. Lesson learned to principal investigators: insist on hardware, and budgets in a timely manner, put the

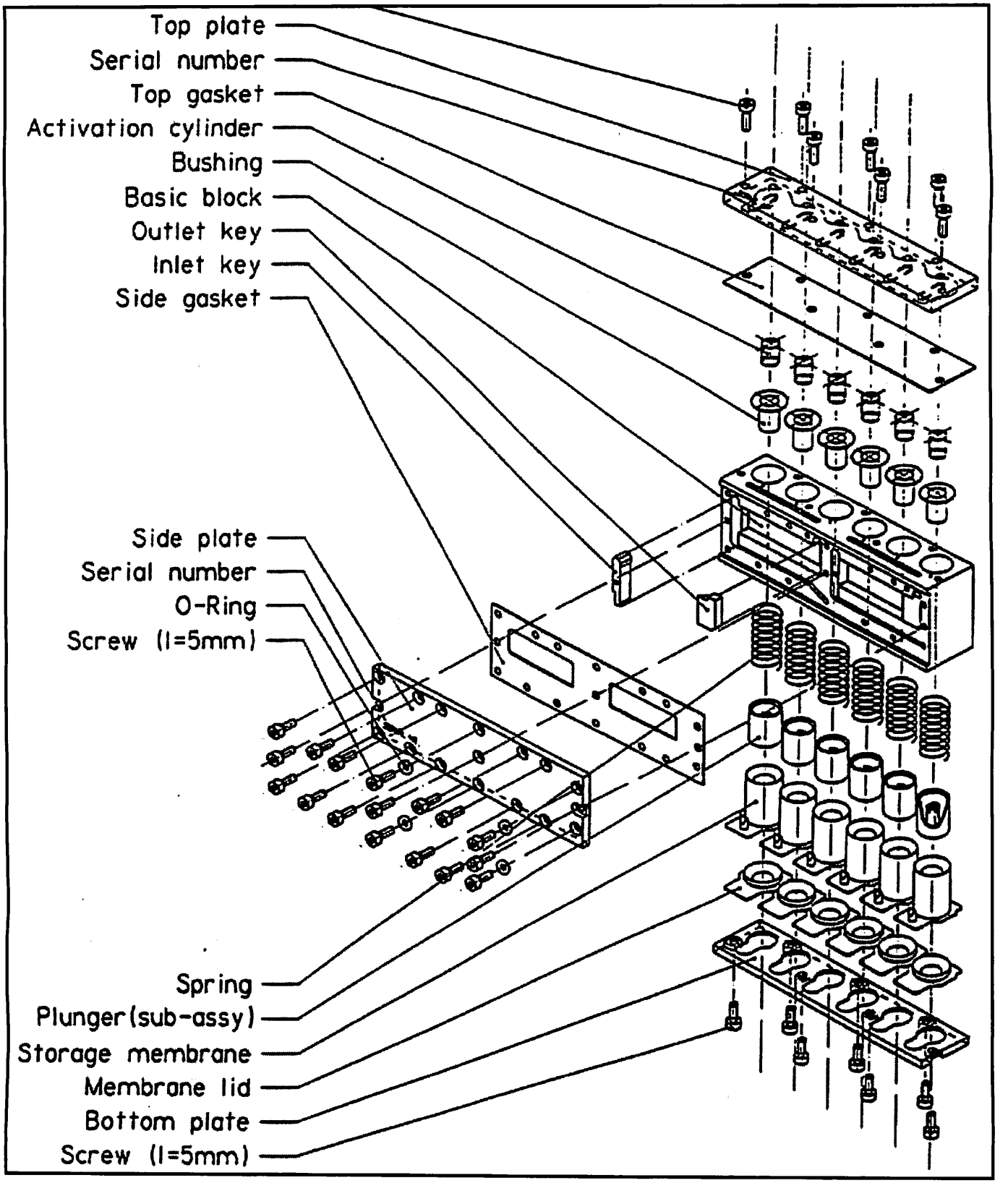

Figure 5. Expanded diagram of the plungerbox components.

individuals with power on your Monday morning call list and call every Monday morning until you have adequate support. Take the opportunity to let them know your progress so they can defend your work.

Lessons learned to all levels of NASA administrators: do not starve your flight science, if you want a quality product, you have to support quality work with appropriate funding and hardware at the appropriate time. Access to microgravity is the most important asset we have in space science and it should be supported. Six months or even twelve months ahead of launch is not nearly enough lead time for good science. Full support should be started at least one year *prior* to setting any of the experimental parameters (hardware, timing, storage of sample, etc.).

CONCLUSION

In summary, lessons learned suggest that you:

1. Beware of launch and landing forces, therefore fly cells in a resting state and collect them prior to landing.
2. Ask for a low shear in-flight 1g centrifuge for control cells.
3. Pick your hardware carefully, make sure its materials are compatible with life and that the hardware does not introduce fluid forces that can turn on gene expression. Also make sure that you can autoclave it for sterilization.
4. Get your hardware early and practice, practice, practice.
5. Make sure that your team is cross-trained; you never know if someone will become ill.
6. Fly enough samples to provide you with data points that will give your statistical significance.
7. Use proper storage and make sure you have appropriate temperature control for your molecule of choice. Bring enough materials to the cape to support up to six launch attempts.
8. Use the KISS! Principal... Keep It Simple Scientists!
9. Get your funding early, start experimental protocols and troubleshooting early. Get all paperwork done well ahead of time.

REFERENCES

1. **Fitzgerald, J., and M. Hughes-Fulford.** Gravitational loading of a simulated launch alters mRNA expression in osteoblasts. *Exp. Cell Res.* 228:168-171, 1996.
2. **Tjandrawinata, R.R., V.L. Vincent, and M. Hughes-Fulford.** Vibrational force alters mRNA expression in osteoblasts. *Faseb J.* 11:493-497, 1997.
3. **Hughes-Fulford, M., and M.L. Lewis.** Effects of microgravity on osteoblast growth activation. *Exp. Cell Res.* 224:103-109, 1996.
4. **Pavalko, F.M., N.X. Chen, C.H. Turner, D.B. Burr, S. Atkinson, Y.F. Hsieh, J. Qiu, and R.L. Duncan.** Fluid shear-induced mechanical signaling in MC3T3-E1 osteoblasts requires cytoskeleton-integrin interactions. *Am. J. Physiol.* 275:C1591-1601, 1998.
5. **Jones, D.B., H. Nolte, J.G. Scholubbers, E. Turner, and D. Veltel.** Biochemical signal transduction of mechanical strain in osteoblast-like cells. *Biomaterials* 12:101-110, 1991.
6. **Hatton, J.P., M. Pooran, C.F. Li, C. Luzzio, and M. Hughes-Fulford.** A short pulse of mechanical force induces gene expression and growth in MC3T3-E1 osteoblasts via an ERK 1/2 pathway. *J. Bone Miner. Res.* 18:58-66, 2003.
7. **Gomez, J., A.Garcia, L. R. B., P. Bonay, A.C. Martinez, A. Silva, , M. Fresno, A.C. Carrera, C. Eicher-Streiber, and A. Rebollo.** IL-2 signaling controls actin organization through Rho-like protein family, phosphatidylinositol 3-kinase, and protein kinase C-zeta. *J. Immunol.* 158:1516-1522, 1997.
8. **Hammond, T.G., E. Benes, K.C. O'Reilly, D.A. Wolf, R.M. Linnehan, A. Taher, J.H. Kaysen, P.L. Allen, and T.J. Goodwin.** Mechanical culture conditions effect gene expression: gravity-induced changes on the space shuttle [In Process Citation]. *Physiol. Genomics* 3:163-173, 2000.
9. **Hughes-Fulford, M.** Signal transduction and mechanical stress. *Sci. STKE 2004*, RE12, 2004.