Inaugural Issue
Volume 1 (June, 2016)

NEXUS

Indian Fertility Society & ORIGIO India Initiative

INTRA UTERINE INSEMINATION (IUI)
Nuts & Bolts

It is a great privilege and pleasure to write this message for the very first e-bulletin of IFS-Nexus. Nexus is the latest endeavor of Indian Fertility Society with full credit for its initiation to our extremely enthusiastic and hardworking Joint Secretary – Dr Pankaj Talwar. The idea of Nexus has been initiated to bridge the gap between ART Clinicians and Embryologists. It aims to enhance the awareness about quality control, basic IVF techniques and lab protocols within the IVF community. On behalf of the Indian Fertility Society I sincerely thank "ORIGIO India Private Limitetd" to partner with us in this great academic endeavor. The first bulletin is dedicated to "IUI Media", which I am sure, all our readers will find very informative and interesting.

My heartiest congratulations to Dr Pankaj Talwar, ORIGIO and the entire team and very best wishes for the future.

Dr. Sohani Verma
President- IFS

Indian fertility Society feels proud and congratulates the editors on the launch of first edition of Nexus e-Bulletin: A journal to enlighten us and broaden the spectra of knowledge in Embryology & Andrology. We take it as our duty and responsibility to train and educate our budding embryologist and infertility specialists right from the basics and this bulletin is a step forward in this direction. It would not only help to disseminate scientific & ethical content but also constantly update everyone with new researches and developments across the globe.

Dr K.D. Nayar
General Secretary-IFS

ART services require complex interaction between the clinical directors , embryologist and technicians for smooth functioning of the establishment. Unfortunately at the present moment we don't have many qualified reproductive laboratory support staff and our young clinicians are also new to this branch having setup the centres with sheer enthusiasm without much of formal training. Majority of our Embryologist too are inexperienced youngsters or relatives of the clinical directors doing on the job training .

Such situations makes ART team members vulnerable to the undesirable situations pertaining to the procurement of the ART media , equipment and using them optimally to obtain desired results . It is the heartfelt desire of Indian Fertility Society to always bring out a bulletin which can ignite thinking process in the readers and guide them to carry on scientific work with firm foundation. **This bulletin has been named NEXUS which means building bridges.** The primary aim is to bridge knowledge gap between the team members, shorten the learning curve and also empower them to interact with the ART agencies selling IUI and IVF related products. Keeping these thoughts in mind IFS has decided to bring out this monthly bulletin on issues which are of common interest to entire team. Our motto is **"Knowledge Empowers"** and we sincerely hope that you would enjoy reading this write up.

In this issue we cover for you all the essential details pertaining to IUI (Intra uterine insemination). As the editor I felt that presenting the facts with aid of pictures and algorithm will convey our thoughts to the busy ART fraternity better and leave long lasting impression in the minds of the readers.

This issue of NEXUS will also be our ready reckoner for all IUI related issues in future pertaining to IUI media accessories procurement, and their optimal safe handling. Please download this issue and file it in folder as the real benefits would be seen after you have gone through all issues at end of one year. Feel free to communicate with us at any point of time and contribute critically.

Your comments would be published in the next bulletin which is titled–Semen analysis: Trouble shooting.

We would formally like to thank my friend Dr Ashish Fauzdar PhD, Scientist I (Embryology) at ESI-PGIMSR Hospital, Basaidarapur, New Delhi who has un-relentlessly and passionately worked towards bringing out this issue from conception to end. This bulletin would not have reached you without his constant support.

We would also like to place on record sincerest thanks to ORIGIO India Private Limited who are helping us in publication of this bulletin and off course we promise that there is **no conflict of interest at any level.**

Wish you happy reading and yes don't forget to file this issue.

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HUMAN TUBAL FLUID MEDIUM (HTF)

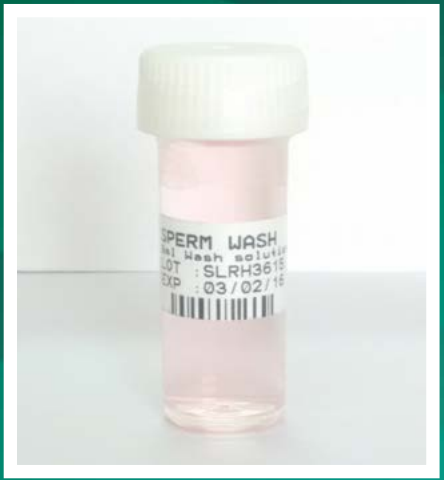


Figure1a: SAR Healthline India (P) Ltd

Figure 1b: Cryo-Genie India (P) Ltd

Figure 1c: Cryocell India (P). Ltd.

SINGLE DENSITY GRADIENT MEDIUM

Figure 2a: SAR Healthline India (P) Ltd

Figure 2b: Cryo-Genie India (P) Ltd

Figure 2c: Cryocell India (P). Ltd.

DOUBLE DENSITY GRADIENT MEDIUM

Figure 3a: SAR Healthline India (P) Ltd

Figure 3b: Cryo-Genie India (P) Ltd

Figure 3c: Cryocell India (P). Ltd.

Figure 1 (a-c) : HUMAN TUBAL FLUID MEDIUM (HTF)

During simple sperm washing technique human tubal fluid (HTF) media is used. HTF is available commercially or can be prepared as per formulation given by Quinn *et al*, 1985. The sperm washing technique with HTF media leads to high yield of spermatozoa from good quality semen sample for intra-uterine insemination (IUI). Human tubal fluid (HTF) media contains HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) that act as a buffering agent by maintaining the physiological pH. The media is supplemented with human serum albumin (HSA), 5mg/ml. The available options for procuring HTF media are given (Figures 1a-c).

Figure 2 (a-c): SINGLE DENSITY GRADIENT MEDIUM

A single density gradient media consist of 80% (v/v) gradient that is composed of colloidal silica coated with silane that has property to separate sperms from other cell types and debris according to their density. The normal progressively motile spermatozoa will swim actively through the gradient material to form a soft pellet at the bottom of the tube. There are various companies that supplies single density gradient kits as shown (Figures 2a-c).

Figure 3 (a-c): DOUBLE DENSITY GRADIENT MEDIUM

A simple two-step discontinuous double density gradient preparation method is most widely applied and typically consists of 45% (v/v) density top layer and 90% (v/v) density lower layer, 100% density gradient is also available commercially. Discontinuous density gradients provide the best selection of good quality spermatozoa from other cell types and debris. It's easier to standardize swim-up technique as results are more consistent. There are number of commercial companies promoting double density gradients kits as shown (Figures 3a-c).

SPERM PREPARATIONS TECHNIQUES:

The choice of sperm preparation technique is decided by the nature of the semen sample. The direct swim-up technique is often used for the normal semen samples. The cases of severe oligozoospermia, tetatozoospermia or asthenozoopermia, density gradient techniques are preferred because of high recovery of motile spermatozoa .

Direct Swim-up Technique: Spermatozoa are selected based on their ability to swim out of seminal plasma into culture medium. The direct swim-up of spermatozoa from semen is the preferred method of separating out the high motile spermatozoa. This technique gives lower yield of spermatozoa than washing and selects sperms on basis of their motility.

Density gradient Technique: This method uses centrifugation of seminal plasma over the density gradients consisting of colloidal silica coated with silane, which separates sperms according to their density. The normal motile spermatozoa with compact head are denser and heavier and will swim actively through the gradient media and thus will settle at the bottom to tube to form a soft pellet.

There are two types of density gradient technique:

Continuous Gradients: In the continuous gradients, there is a gradual increase in density from the top of the gradient to its bottom.

Discontinuous Gradients: In discontinuous gradient there are clear boundaries between lower and upper gradients. The most widely used discontinuous density gradient preparation includes 45% (v/v) density (top layer) and 90% (v/v) density (lower layer).

STORAGE TEMPERATURE OF THE IUI MEDIA

Figure 4a : Media Ampoules are recommended to be stored between 2-8 °C

Figure 4b : All IUI Media are stored in original container in Drug Refrigertors

Figure 4c : Media ampoules are simultaneosly warmed at 37 °C in Dry Bath Warmer

HOW TO OPEN THE MEDIA VIAL

Figure 5a: Firmly hold the base of the vial with two fingers and apply pressure on the tip of the vial with fulcrum at the neck area (blue ring)

Figure 5b: Wrap the tip of the vial in gauge piece before applying the pressure, to avoid accidental injury. If properly done the vial will break along the blue line

Figure 5c: Show the recommended ampoule opener (Arian Biotech System) to avoid sharp injuries & media spillage

HOW NOT TO OPEN THE MEDIA VIAL

Figure 6a: Use of traditional methods of breaking glass ampoule by use of scalpel handle, spatula or scissors are not recommended

Figure 6b: Scattered broken pieces of glass ampoule due to wrong practice of breaking vial with force

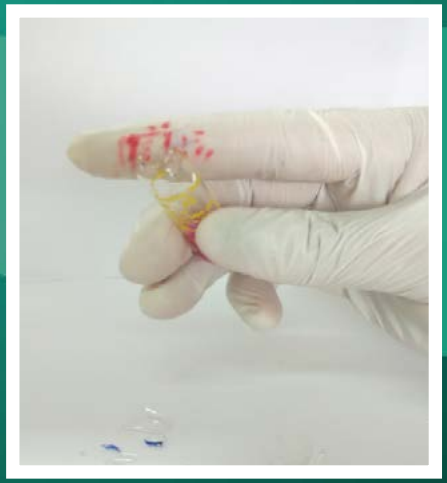


Figure 6c: Sharp injury due to wrong practice of ampoule breaking

Figure 4 (a-c): STORAGE TEMPERATURE OF MEDIA

The storage temperature for IUI media is between 2-8ºC (Figure 4a & 4b). One should remember that IUI media are warmed at 37ºC in dry bath warmer before utilizing them for sperm preparation. This would avoid cold shock to the spermatozoa and also eliminate condensation on the surface of vial (Figure 4c). Since all IUI media contains HEPES that acts as a buffering agent and maintains the pH between 7.2-7.4, they don't require CO_2 gas incubators for equilibration of the media.

Figure 5 (a-c): HOW TO OPEN THE MEDIA VIAL

Ampoule sharp injuries are a serious occupational health and safety hazard. It's important to highlight the correct method of opening the ampoule. Before opening the ampoules, they are cleaned with 70% ethanol or available IVF grade cleaning agent (Figure 5a & 5b). We recommend the use of various ampoule openers commercially available or multifunctional ampoule opener/cutter (from Arian Biotech System, unit price 700 INR). The ampoule opener fit to any size of glass ampoule and ensure protection to the health care staff (Figure 5c).

Figure 6: HOW NOT TO OPEN THE VIAL

Breaking glass ampoule by use of scalpel handle, spatula or scissors is not recommended. However lot of laboratory personnel's have habit of breaking them using these traditional methods (Figure 6a). These methods have potential health hazard to laboratory technician. The uneven glass surfaces of ampoule or scattered glass pieces on floor area can lead to sharp injuries (Figures 6b & 6c).

Figure 7: Shows cleaning of ampoules for maintaining the sterility.

Figure 7: CLEANING OF MEDIA VIALS

All the media products are supplied 'STERILE' and hence aseptic techniques should be used for cleaning and handling of media ampoules before patient use. The exterior is thoroughly cleaned with sterile gauze piece soaked with 70% ethanol, quaternary ammonium chloride or available hypochlorous chloride cleaning solution specific for IVF laboratories (Figure 7).

Figure 8: EXPIRY PERIOD & BATCH NUMBER

All media ampoules back label should be carefully checked for expiry date, batch & lot number before patient use (Figure 8). Any media ampoule beyond expiry date should be immediately discarded. Batch / lot number is documented for Quality Control purpose.

Figure 8: All media ampoule are checked for expiry date & batch number as written on the label of ampoule before use

Figure 9: Dispensing of the media using sterile transfer pipette

Figure 9: HOW TO DISPENSE THE MEDIA

After carefully opening media ampoule as described above (Figure 5a-c) dispense media using the sterile transfer pipettes to the centrifuge tube provided in the IUI accessory kit. Try avoiding formation of air bubbles while transferring the media from media ampoule to the centrifuge tube (Figure 9).

Table 1: Commercially available IUI Readymade kits

IUI Media & Consumable Suppliers	**SAR Health Line (P) Ltd** Ph: +91 11 - 26494178, Mob: +91 9871244044 Web: www.sarhealth.com	**Cryo-Genie India (P) Ltd** Ph: 011-32223643, Mob : +91-9871291841 Web: www.cryogenie.com	**Cryocell India (P). Ltd.** Ph: +91-11-22543201, Mob : +91-9310112796 Web: www.infertech.in
Sperm Wash HTF Media (1X 5 ml HTF Wash Media)	267.50	110	100
Double Density Gradient Kit (1 X 1 ml 45% Gradient Media) (1 X 1 ml 90% Gradient Media) (1 X 5 ml HTF Wash Media)	397.50	405	405
Single Density Gradient Kit (1 X 2 ml 80% Gradient Media) (1 X 5 ml HTF Wash Media)	342.50	375	380
Accessory Pack (Transfer Pipettes x 2) (Centrifuge tube x 2)	32	30	36
IUI Cannula (17 cm)	50	90	50
Semen Collection Container	8	10	11
Cost per patient (Sperm Wash HTF Media & Accessories)	358₹ (5.2 $; 4.65 €)	240₹ (3.10$;2.77 €)	197₹ (2.91 $;2.60 €)
Cost per patient (Double Density Gradient Kit & Accessories)	488₹ (7.12 $;6.37 €)	535₹ (7.46 $; 6.67€)	502₹ (7.41 $;6.63 €)
Cost per patient (Single Density Gradient Kit & Accessories)	433₹(6.31 $;5.64 €)	505₹ (7.02 $; 6.27€)	477 ₹ (7.05 $;6.30 €)

** Per unit prices in INR excluding local taxes & freight charges as applicable. Prices in Dollar & Euro may vary depend on the exchange rates as applicable*

IUI media is available as single patient kits from the major Indian suppliers. There are also sperm preparation / washing and gradient media available from global suppliers such as **Cook, Origio, Vitrolife** etc., though not as single patient kits.

PROTOCOL'S FOR IUI SAMPLE PREPARATION

SINGLE DENSITY GRADIENT TECHNIQUE

- Transfer 2 ml of single density gradient solution to a conical tube. Layer liquefied semen sample over the media. Centrifuge at 1200 rpm for 15 minutes.
- Carefully discard the supernatant that contains seminal plasma, inter-phase and single density gradient media layer.
- Add 1 to 2 drops of HTF wash media over the pellet and resuspend the pellet in the media then shift the suspension to a new tube.
- Add 4 ml of HTF wash media to the suspension & mix well.
- Centrifuge the tube at 1200 rpm for 5 minute.
- Discard the supernatant.
- Resuspend the pellet in 0.5 ml of HTF wash media.
- Do post wash examination on a small aliquot to calculate the yield.
- Alternately we can layer the pellet with 1 ml of HTF and collect 0.5-0.8 ml of supernatant for the IUI procedure.
- Sample is ready for IUI

DOUBLE DENSITY GRADIENT TECHNIQUE

- Transfer 1ml 90% density gradient media ampoule to conical centrifuge tube.
- Carefully layer 1 ml of 45% density gradient solution over the 90% solution without mixing.
- Transfer liquefied well mixed semen sample and overlay on top of media.
- Centrifuge for 15 minutes at 1200 rpm.
- After centrifugation carefully remove the supernatant without disturbing the pellet.
- Add 1 to 2 drops of HTF wash solution over the pellet. Shift to the suspension to the new tube.
- Add 4 ml of HTF wash solution & mix well.
- Centrifuge the tube again at 1200 rpm for 5 minutes.
- Remove the supernatant.
- Resuspend the pellet in 0.5 ml of HTF wash media.
- Do post wash examination on a small aliquot & to calculate the yield.
- Sample is ready for IUI

WASH SWIM UP TECHNIQUE

- Transfer ~3 ml semen sample in conical tube and add 5 ml of HTF media. Mix well & centrifuge at 1200 rpm for 10 minutes.
- Remove supernatant without disturbing the pellet.
- Gently layer 2 ml of HTF media on the pellet and incubate for 20-30 min at 45° angle at 37°C incubator without CO_2.
- Collect 0.8-1 ml of the swim-up and transfer to the new tube.
- Take a small drop for post wash analysis.
- Sample is ready for IUI

NEAT SEMEN SWIM UP TECHNIQUE

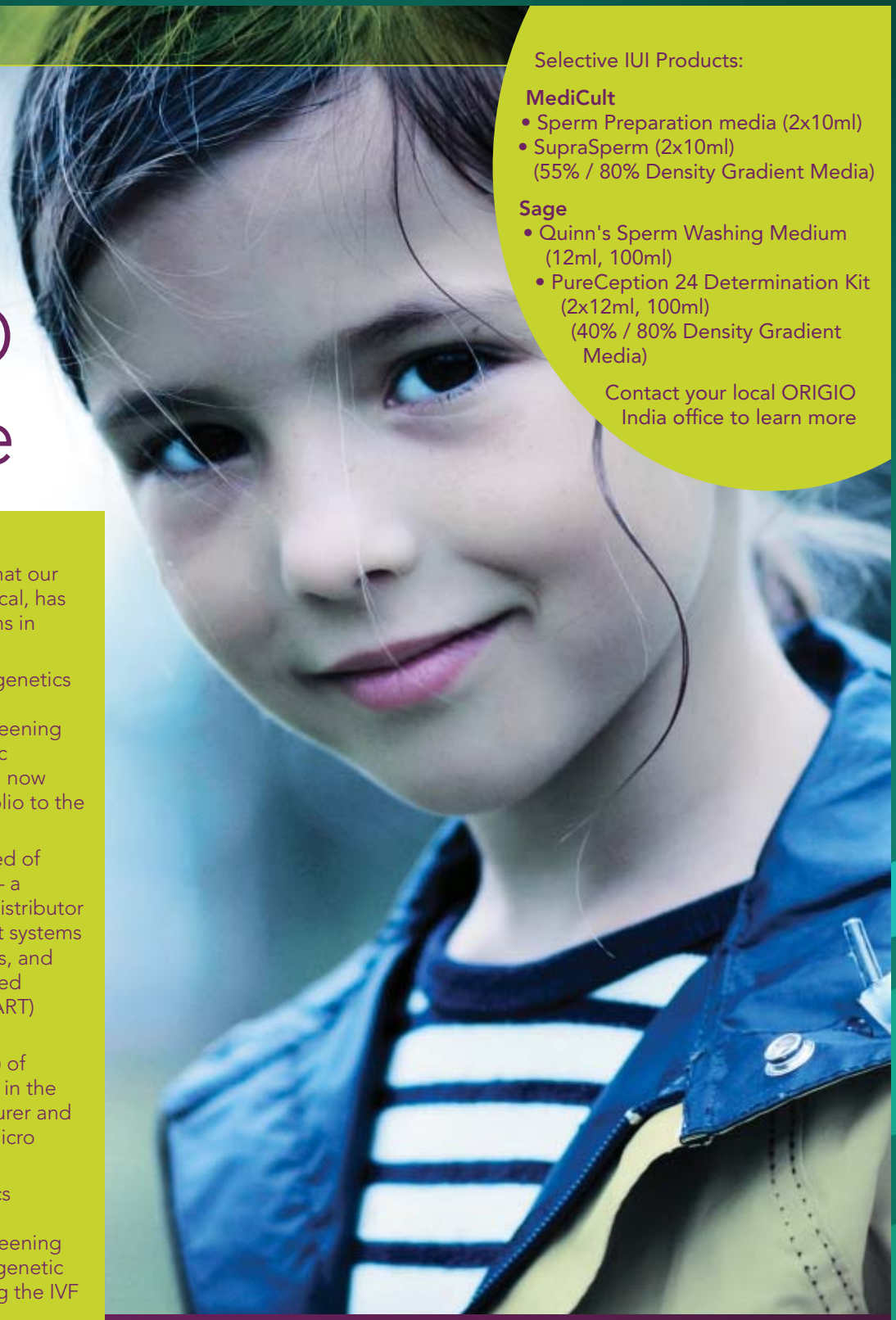
- Add complete liquefied semen sample in a conical tube.
- Layer 2 ml of HTF media on top of the semen gently.
- Incubate at 45° for 20-30 minutes at 37°C, in non CO_2 condition.
- Aspirate the supernatant from the tube and shift to the new conical tube.
- Centrifuge for 5 minutes at 1200 rpm.
- Discard the supernatant and add 0.5 ml of HTF media to the pellet and resuspend.
- Do post wash examination on a small aliquot to calculate the yield.
- Sample is ready for IUI.

Conclusion:

Intra Uterine Insemination (IUI) is a simple technique of Assisted Reproductive Technology (ART) for treating infertility by artificial insemination. It's important to separate human spermatozoa from seminal plasma through any one of the sperm preparation technique (swim-up, swim & wash, or density gradient) to yield a final preparation containing high percentage of morphologically normal and motile cells, free from debris, non germ cells and dead spermatozoa. Laboratory personnel should follow the recommended scientific methods as highlighted in this issue for processing of samples for best possible results.

Glimpse of the NEXUS volume 2 (July 2016) : Semen analysis

- WHO guidelines
- Semen analysis simplified
- Do's and dont's of Semen analysis
- Sperm counting chambers
- Sperm function test and morphology



ORIGIO – for life

Selective IUI Products:

MediCult
- Sperm Preparation media (2x10ml)
- SupraSperm (2x10ml) (55% / 80% Density Gradient Media)

Sage
- Quinn's Sperm Washing Medium (12ml, 100ml)
- PureCeption 24 Determination Kit (2x12ml, 100ml) (40% / 80% Density Gradient Media)

Contact your local ORIGIO India office to learn more

Announcement

We are excited to announce that our parent company, CooperSurgical, has made the following acquisitions in recent months:

- Reprogenetics, the largest genetics laboratory specializing in preimplantation genetic screening and preimplantation genetic diagnosis, the company can now offer a total solutions portfolio to the ART market.
- Research Instruments Limited of Cornwall, United Kingdom – a leading manufacturer and distributor of equipment, management systems (RI Witness System™), lasers, and micropipettes for the Assisted Reproductive Technology (ART) market.
- The Pipette Company (TPC) of Adelaide Australia is known in the ART industry as a manufacturer and distributor of high quality micro pipettes.
- Genesis Genetics, a genetics laboratory specializing in preimplantation genetic screening (PGS) and preimplantation genetic diagnosis (PGD) used during the IVF process.
- K-Systems Kivex Biotec A/S, market leader in developing, manufacturing and distribution of innovative and expertly-designed equipment to IVF clinics.
- Assets of Recombine Inc., a clinical genetic testing company specializing in carrier screening.

Learn more at **www.origio.com**

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