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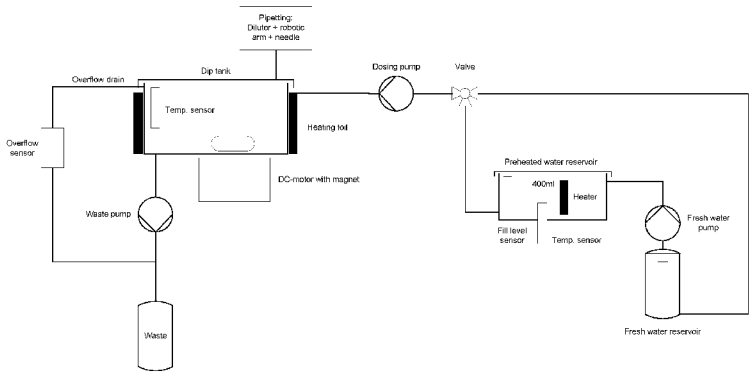

Figure 3

(57) Abstract: In a system and method for washing and target retrieval of tissue samples mounted on microscope slides for use in immonistochemical procedures and cytology, each of a plurality of dip tanks comprises a housing and a holding structure for holding a single microscope slide in a fixed position. A fluid inlet may be provided at an upper end of the housing structure, and an outlet may be provided at the lower end of the housing structure. An agitator is provided for agitating fluid in the tank compartment. The system may comprises a single reservoir of liquid, a dosing pump for pumping a controlled dosage of liquid from the reservoir to the fluid inlet of each of the dip tanks, a preheater for heating liquid pumped from the reservoir to the dip tanks, a fluid control system configured to allow liquid pumped from the reservoir to the dip tanks to selectively bypass the preheater, a housing heater for heating at least a portion of the housing of each of the dip tanks, an overflow drain, associated with each one of the dip tanks, a drive for driving the agitator within the dip tank, and a waste reservoir for liquid.

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A SYSTEM AND A DIP TANK FOR WASHING AND TARGET RETRIEVAL OF TISSUE SAMPLES MOUNTED ON MICROSCOPE SLIDES

Technical Field

The present invention relates to a system and a dip tank for washing and target retrieval of tissue sample mounted on microscope slides. The system and dip tank according to the invention may be employed in an automated instrument for immunohistochemical staining and cytology using different antibodies or molecular probes in diagnosis of various diseases.

Background of the invention

Preparation of tissue samples prior to sectioning and mounting on microscopes slide and target retrieval methods will be briefly described below. In particular that the specific target retrieval and staining methods has a strong influence on the resulting outcome of the analysis.

Tissue is often fixed and embedded and cast into blocks before sectioning.

Tissues may be fixed by either perfusion with or submersion in a fixative, such as an aldehyde (such as formaldehyde, paraformaldehyde, glutaraldehyde, and the like). The most commonly used fixative in preparing samples for IHC is formaldehyde, generally in the form of a formalin solution (4% formaldehyde in a buffer solution, referred to as 10% buffered formalin).

Other fixatives include oxidizing agents (for example, metallic ions and complexes, such as osmium tetroxide and chromic acid), protein-denaturing agents (for example, acetic acid, methanol, and ethanol), fixatives of unknown mechanism (for example, methanol, ethanol, propanol, mercuric chloride, acetone, and picric acid), combination reagents (for example, Camoy's fixative, methacam, Bouin's fluid, B5 fixative, Rossman's fluid, and Gendre's fluid), microwaves, and miscellaneous (for example, excluded volume fixation and vapor fixation).

Additives may also be included in the fixative, such as buffers, alcohols, detergents, tannic acid, phenol, metal salts (for example, zinc chloride, zinc sulfate, lanthanum and lithium).

Paraffin is used in the histochemical art for embedding or otherwise supporting biological samples for histological or other analyses. When casted in blocks, the sectioning of the sample is possible. Examples of embedding medium include, but are not limited to, wax,

paraffin, paramat, paraplats, peel away paraffin, tissue freezing medium, cryonic gel, OCT™ ("Optimum Cutting Temperature") embedding compound, Polyfin™, and polyester wax.

The combination of formalin fixed and parafin embedded tissue is referred to as FFPE tissues.

The FFPE tissue blocks containing the material to be analyzed are first trimmed and then cut into thin sections on a manual or automatic microtome. The 2-10 micrometer thin sections are collected on a water bath and placed on labeled microscope slides. The slides are used for the primary or advanced staining procedures or intermediately stored. In the advanced staining procedure, including IHC, the first procedural steps conducted on the FFPE tissue on slides are baking and dewaxing of the tissue and for most tissues to be stained by the IHC or ISH methods, a target retrieval step is included, also referred to as epitope retrieval, target antigen retrieval or target unmasking.

Target retrieval (TR) and antigen retrieval (AR) have also been used in other applications than IHC, such as PCR and in situ hybridisation.

In general, for FFPE tissue, the target retrieval process breaks the protein cross-links caused by the formalin fixation process and unmasks the antigens and epitopes, thus enhancing the staining intensity of the applied antibodies or molecular probes.

Unfortunately, not all targets can be target retrieved with the same protocol. Also, different levels of fixation or different fixation methods and fixatives demand different target retrieval procedures in order to facilitate specific and efficient staining.

The most common target retrieval method is treatment of the sample in a suitable buffer at elevated temperature, typically 90-105 °C for 10-60 minutes. The process is referred to as heat induced epitope retrieval or HIER or heat induced antigen retrieval (HIAR).

HIER was first introduced by Shi et al. in 1991 using distilled water, zinc chloride and lead thiocyanate as incubation media and a microwave oven as heat source. It is the heat which speeds up the hydrolysis reaction and subsequently makes the antigen accessible for e.g. specific antibodies.

A large number of different heat sources have been used for the process, from the initial microwave oven to regular ovens, water baths, autoclaves, steamers and pressure boilers.

It has been shown that different heating methods yield similar retrieval effects if the heating times are adjusted.

HIER can be obtained by many methods by heating the sample on the slide in a target retrieval buffer, for example while the slide is in a horizontal position, vertically in dip tanks at atmospheric pressure, in microwave ovens or in pressure cookers. The efficiency of HIER is a function of temperature, time, pH and chemical composition of the buffer. Temperature and time are inversely related: 120°C in a pressure cooker for 5-10 min. roughly corresponds to 100°C in a microwave oven for 20 min. or 60°C in an incubator for 24 hours. High temperature or prolonged heating can, however, cause damage to the morphology, especially if the tissue is weakly formaldehyde fixed or partial detachment of especially fatty tissue types from the microscope slide.

This is basically in accordance with the Arrhenius relationship between reaction speed and temperature. A general rule of thumb is that 10 ^{0}C increase in temperature during TR will speed up the reaction 2-4 times. Consequently, a slight variation in temperature can significantly change the outcome of the HIER procedure.

A majority of epitopes are retrieved with HIER treatment at high pH (e.g. pH 9, TRIS, EDTA). In general, the high pH HIER seems to give a subsequent higher staining intensity than e.g. low pH methods, but sometimes at the cost of changes in cell and tissue morphology.

Numerous solution mixtures can be used for HIER, including Tris(hydroxymethyl)aminomethane (TRIS), urea, EDTA, citrate and saline buffers. Citrate pH 6 and TRIS with EDTA at pH 9 are the most common. Reagents for controlling the pH of the solution can be chosen from a wide range of buffers such as TRIS, citrate, phosphate, glycine or Good buffers, such as BES, BICINE, CAPS, EPPS, HEPES, MES, MOPS, PIPES, TAPS, TES or TRICINE, metal chelating compounds like EDTA or EGTA, microbial preservatives like azide, glycerol, glycols, PEG, polar organic solvents or ionic or non-ionic surfactants like NP40 or Tween20/80. Other HIER systems use solutions of citraconic anhydride (CCA) or pure distilled water.

Some epitopes are best retrieved at low pH (e.g. citrate pH 6). Examples include Prion Protein, clone 3F4, epithelial related antigen, clone MOC-31 and to some degree also e.g., epithelial antigen, clone Ber-EP4; CD31, clone JC/70A; glycoprotein 200, clone 66.4.C2; and epithelial related antigen,clone MOC-31.

Other epitopes are best retrieved by proteolytic enzyme digestion. This is done at near room temperature. Pronase, pepsin and trypsin are the enzymes most frequently used. Pepsin

seems particularly useful for extracellular epitopes (for example collagen IV, laminin). Other examples of antibodies for which the epitopes are preferably retrieved using proteolysis include cytokeratin (CK) 8/7, clone Cam 5.2; prostate specific antigen, clone 28/A4; and collagen IV, clone CIV 22.

A few epitopes are best retrieved by a procedure including both proteolytic enzyme digestion and HIER. Examples include Collagen VI, VI-26, clone VI-26, Calpain clone 12A2 and Spectrin, clone R8C2/3D5. Some epitopes are apparently demasked equally well by HIER and proteolysis. Important differences may exist, however. Thus, using either of the methods S-100beta protein may be demasked with the same efficiency in nerves, whereas only HIER allows proper detection of S-100beta in some epithelia and striated muscle. Cytokeratin 20, clone Ks20.8 gives a false positive staining in some epithelia after proteolysis but not after HIER. In some cases, where both heat and proteolysis provides good epitope retrieval, the latter method may require a higher Ab concentration to give an optimal result.

Some epitopes in FFPE tissue can be stained without target retrieval or with a much milder treatment than others, resulting in a better preserved morphology. Examples include Glucagon, clone A0565 and Growth hormone, clone MU028-UC.

Updated lists of epitopes, antibody clones and best practice target retrieval procedures are available from IHC quality and standardization organizations, such as Nordic Immunohistrochemical Quality Control (NordicQC), College of American Pathologists (CAP) or United Kingdom National External Quality Assessment Service (UK-NEQAS).

Frozen tissues or cryo samples are normally not target retrieved by HIER, as they are not covalently fixed or only slightly fixed and therefore risk disintegrating and losing the morphology.

A systematic IHC diagnosis of a tissue sample uses the staining pattern of different target specific markers on several slides organized in so-called antibody panels. Positive or negative staining patterns for each antibody are used in diagnostic algorithms to extract the diagnostic result based on experience and statistics. Algorithms are used, for example, for search of the primary cancer site, to rule out non-carcinoma, and for tumour subclassification. Panels are organized in groups, for example, identifying tumours of unknown origin, or differentiating haematolymphoid and non-haematolymphoid neoplasms. The panels can be organized in several smaller rounds of analysis, making the classification narrower for each round of analysis. Alternatively, the panel can be large enough to give the diagnosis in the first round.

It will be understood from the foregoing that in order to perform a correct diagnosis based on groups and panels of antibodies, one will often need to treat the tissues mounted on slides with different target retrieval procedures and stain using different antibodies.

This makes sorting of slides necessary if the automated instrument cannot perform different target retrieval protocols for each slide in a group.

In the following the most relevant target retrieval prior art is described in more detail.

A widely used semimanual instrument is the "Automated Dewaxing and Epitope Recovery Device for Lab Vision Autostainers" (Thermo Fisher / Lab Vision), referred to as the PT module. It is a simple heated dip tank holding two racks of 12 slides each, i.e. all slides receive the same treatment.

The total TR buffer volume is about 1.5 liter and there is no agitation of the buffer during processing. The typical heat up time is 20 minutes and the cool down time about the same. The temperature is controlled by a vapor switch, which detects the boiling and condensing water. When triggered, the temperature is turned down about 2-3 °C and kept there during the processing. The tank is cooled down by turning off the heating and by passing air from a ventilator past the tank. This normally takes 15-20 minutes.

As with all dip tank protocols, one can often only remove the slides when the temperature is reduced to approximately 40-50 °C, to prevent rapid dry out of the tissue when exposed to the air.

The advantage of the PT module is the simplicity of the instrument and the possibility to do "3-in-1" procedures, i.e. dewaxing, rehydration and target retrieval in one step. It should be noted that the operator will subsequently need to manually rinse the slide with preheated water to remove detergent and paraffin residues after the TR process.

The other major drawback is the manual operation, slow heat up and cool down times and the fact that all slides are treated with the same protocol, making sorting according to target retrieval protocol necessary.

Similar dip tank arrangements have been described incorporated into automated stainers, including US 2006/0088928 and US2011136135A1.

In both stainer instrument designs the heated dip tanks process multiple slides with the same TR protocol.

Another general disadvantage of shared dip tanks is the risk of so-called tissue floaters. That is, dislodged tissue migrating from one slide to another slide in the same dip tank, subsequently resulting in wrongful diagnosis.

The microwave oven technique pioneered by Biogenex is a simple variation of the dip tank method. Typically, slides are batchwise immersed into a Joplin jar placed in a conventional microwave oven. Basically, the temperature is still under the boiling point of water on average. It is debated if the temperature is locally higher and the chemistry (hydrolysis) is helped by the applied microwave irradiation.

A variation of the above method is treatment in a pressure cooker. The target retrieval procedure is conducted at elevated pressure and temperature (100-115 °C) using conventional kitchen pressure cookers originally designed for cooking rice (David Tascha, BioCare Medical, US 6580056). The advantage is the higher temperature and thus shorter target retrieval process time. The drawback is the potential loss of morphology due to the higher temperature and the long heat up and cool down time, which almost makes the overall process time equivalent to the other techniques.

A variation of the Biocare pressure cooker device with a better pressure control is described by Ljungmann et al, WO2005/057180A1, "An Apparatus for treatment of tissue specimens".

In an automatic instrument used in a routine laboratory, atmospheric pressure is preferred to reduce the design and control complexity. Further, pressurized tanks are associated with some specific safety issues, which make them difficult to operate and incorporate into larger instrument systems.

In all of the above described systems, batches of slides are treated with the same protocol in a heated and static dip tank arrangement.

A different general method of target retrieval incorporated into automated stainers treats the slides individually.

The slides are heated from below by a heating plate and a small amount (<1-2 ml) of TR buffer is placed over the sample. This is done in a near horizontal position in a stainer instrument.

Examples include the BenchMark stainer (Ventana/Roche), which uses a liquid oil cover slip and frequent replacement of the TR buffer below to prevent drying out of the tissue during processing. The disadvantage of such procedures include their extended process time due to the relatively low temperature, estimated to be about 80-90 °C, and the challenge of ensuring a homogeneous temperature distribution across the slide. Especially the contact between the slide and the heating plate is critical for obtaining reproducible target retrieval results.

A similar system is described in WO2008/095501A1, though without cover slips. A formulation with the highly hygroscopic glycol and other components reduces the evaporation of water at high treatment temperature near the boiling point of water. The disadvantages are the frequent replacement of TR buffer and difficulties in controlling the temperature.

Several stainer designs use different types of slide cassettes or similar systems for protecting against dry out.

Examples include the automated target retrieval system used in the Bond stainer (Leica Microsystems, Melbourne). The stainer utilizes a solid cover tile system instead of a liquid cover slip to protect against evaporation. The microscope slide is heated by a heating block from below.

Yet another target retrieval system incorporated into a stainer is described in US6830292. The slide is placed in a cassette together with a metal plate. By electric induction, the plate is heated and the heat transferred to the slide and target retrieval buffer.

One advantage of the systems above is the fast heat up and cool down due to the low volume.

One drawback of the above prior art systems incorporated into automated stainers and using close-to horizontal slides, small buffer volumes, static conditions and heating plates is the difficulty of controlling the temperature and also a long process time, as the temperature is to be kept somewhat below the boiling point of water due to the risk of evaporation.

Objects of the invention

It is hence an object of embodiments of the invention to provide a system, which overcomes the disadvantages of the prior art. It is a further object of embodiments of the invention to provide a system, which allows for accurate temperature control of a liquid, such as a target retrieval buffer, while or before tissue samples on microscope slides are subject to target

retrieval. It is a further object of embodiments of the invention to provide a system, which is fast. It is a further object of embodiments of the invention to provide a system, which renders it possible to treat individual tissue samples mounted on respective slides with individual target retrieval procedures.

Summary of the invention

In a first aspect, the present invention provides a system for washing and target retrieval of tissue samples mounted on microscope slides, comprising a plurality of dip tanks, each of which comprises:

- a housing structure and, within the housing structure, a holding structure for holding a single one of the microscope slides in a fixed position relative to the housing structure; the housing structure defining a tank compartment having an upper open end for receiving the microscope slide, and a lower essentially closed end opposite to the open end;
- a fluid inlet, which is preferably arranged at or near the upper end of the housing structure;
- a fluid outlet, which is preferably arranged at or near the lower end of the housing structure;
- an agitator for agitating fluid in the tank compartment;

said system further comprising:

- a reservoir of liquid;
- a dosing pump for pumping a controlled dosage of liquid from the reservoir to the fluid inlet of each of the dip tanks;
- a preheater for heating liquid pumped from the reservoir to the dip tanks;
- a fluid control system configured to allow liquid pumped from the reservoir to the dip tanks to selectively bypass the preheater;
- a housing heater for heating at least a portion of the housing of each of the dip tanks;
- an overflow drain associated with each one of the dip tanks;
- a drive for driving the agitator within the dip tank;
- a waste reservoir for liquid;
- at least one conduit connecting the fluid outlet of the dip tanks to the waste reservoir.

In a second aspect, the present invention relates to a dip tank itself, i.e. a dip tank for washing and target retrieval of a tissue sample mounted on a microscope slide, comprising:

- a housing structure and, within the housing structure, a holding structure for holding a single microscope slide in a fixed position relative to the housing structure; the housing structure defining a tank compartment having an upper open end for receiving the microscope slide, and a lower essentially closed end opposite to the open end;
- a fluid inlet, which is preferably arranged at or near the upper end of the housing structure;
- a fluid outlet, which is preferably arranged at or near the lower end of the housing

structure;
- a housing heater for heating at least a portion of the housing structure;
- an agitator for agitating fluid in the tank compartment.

It will be appreciated that the dip tank of the second aspect of the invention is suitable for use with the system according to the first aspect of the invention, i.e. as one of the dip tanks of the first aspect of the invention.

In a third aspect, the invention provides a method for washing and target retrieval of tissue samples mounted on microscope slides, comprising a plurality of dip tanks, each of which comprises:
- a housing structure and, within the housing structure, a holding structure for holding a single one of the microscope slides in a fixed position relative to the housing structure; the housing structure defining a tank compartment having an upper open end for receiving the microscope slide, and a lower essentially closed end opposite to the open end;
- a fluid inlet and a fluid outlet;
- an agitator for agitating fluid in the tank compartment;
said method comprising:
- supplying a controlled dosage of liquid from a reservoir of liquid to the fluid inlet of each of the dip tanks;
- controlling the supply of liquid to allow liquid pumped from the reservoir to the dip tanks to selectively pass through a preheater for heating the liquid, and to allow liquid pumped from the reservoir to the dip tanks to selectively bypass the preheater;
- heating at least a portion of the housing of at least some of the dip tanks;
- agitating the liquid in the dip tanks;
- conveying the fluid from the dip tanks to a waste reservoir.

Thanks to the provision of the preheater, the liquid, typically water, a target retrieval buffer or a washing liquid, entering the dip tanks may be at an elevated temperature, e.g. about 65-70°C, and hence the time required in order to heat up the target retrieval buffer to an appropriate processing temperature is reduced in comparison to systems, which include no preheater. The preheater may also be used for preheating a washing liquid, which may or may not be an amount of target retrieval buffer also used for washing. Hence, washing may be carried out at the preheated temperature in order to ensure efficient washing of remaining embedding media or other deposits. The preheater may, as previously mentioned be configured to heat the target retrieval buffer liquid and/or the washing liquid to a temperature of about 65-70°C, or it may be configured to heat the liquids to the processing temperature, which is typically about 90-99°C, such as about 97-99°C, such as about 98°C. The preheater may comprise a heat exchanger, in which heat from an element heated by e.g.

an electrical heater, is transmitted to the liquid by convection. It will hence be appreciated that the preheater contributes to ensuring swift operation of the system in that the time between two subsequent target retrieval procedures is reduced.

The preheater may comprise a reservoir for the liquid, so as to allow an amount of liquid for a second batch of microscope slides to be preheated, while a first batch of microscope slides is being processed. In preferred embodiments of the invention, the reservoir for the liquid may be configured to contain at least 100 ml of liquid, such as at least 200 ml, or at least 400 ml, or about 300 – 500 ml, such as about 400 ml.

The preheater may comprise a tank including a heating element, such as a boiler, from which heated liquid is pumped to the dip tanks. Alternatively, the preheater may comprises an in-line heating element for continuously heating liquid passing therethrough.

The agitator contributes further to swift operation in that liquid in the dip tank is efficiently mixed.

The agitator´s mixing action efficiently redistribute the heat and reagent concentrations during washing, warm up, processing and cool down operations.

The housing heater of each of the dip tanks may be individually controllable in order to allow tissue samples mounted on respective slides to be treated individually. The term housing heater should be understood broadly to encompass any heating element capable of or configured to heat a wall of the housing, including an interface for connecting the housing to an external heating element. The housing heater may alternatively or additionally include an element, such as a heating coil within a compartment of the dip tank for heating liquid within the dip tank.

The housing heater further contributes to fast operation of the system given that the microscope slides are held in individual dip tanks, i.e. each dip tank holding a single microscope slide. Hence heat dissipated from the housing heater is required to heat only a relatively small amount of liquid, and the time necessary for heating up is accordingly correspondingly short. Likewise the time needed for cooling the microscope slide following the target retrieval procedure is shortened.

It will hence be understood that the dip tank of the system of the present invention is configured to hold one and only one microscope slide at a time.

In order to maximize the capacity of an apparatus embodying or including the system of the invention, the slides may be oriented in an upright orientation, e.g. vertically, when held in the dip tank.

In the system of the present invention, the plurality of dip tanks may be lined up side by side, or they may be arranged in a matrix or pie configuration. In one embodiment, the dip tanks are lined up side by side, and the microscope slides are likewise held in a frame side by side. In such an embodiment, labels on the microscope slides are easily readable, and the space occupied by the system is mimimized.

During operation, each slide may be submerged in liquid in the dip tank only to such an extent that a label area of the slide is not submerged in the liquid. Accordingly the label may be read while the slide is submerged, and possible damage to the label area or contamination thereof caused by the liquid may be prevented.

Generally, the system according to the present invention allows tissue samples mounted on respective slides to be treated individually, i.e. by individual target retrieval procedures, as the temperature of each dip tank is individually controllable. Moreover, conduits connecting the dosing pump to the fluid inlet of the dip tanks may comprise control elements, such as valves, for individually controlling the supply of liquid to each of the dip tanks, and likewise valves and a waste pump may be provided for individually controlling emptying of each one of the dip tanks. In such case, one dip tank may be emptying, while a target retrieval procedure is carried out in another one, and while a yet further dip tank is being filled. In preferred embodiments of the invention, all dip tanks are filled and emptied simultaneously for ease of control.

In one embodiment of the invention, a plurality of slides are mounted on or supported by one rack or frame, and yet each individual slide is held in its own dip tank. Handling of a plurality of slides may thus be facilitated, as a plurality of slides may be loaded together into an apparatus incorporating or embodying the system according to the invention, i.e. with the plurality of slides in the rack or frame (also referred to as "case"), and unloaded together. Further, the number of handling operations is minimized if the plurality of slides are held together in a rack or frame. Yet, each individual slide is submerged into its own dip tank to benefit from the effects and advantages of the invention.

The system and dip tank of the present invention is suited for histochemistry and cytology. In particular, the present invention is suited for washing and target retrieval of tissues mounted on microscope slides.The dip tanks can be arranged in arrays or clusters in an automatic

stainer. The heated dip tank can be filled with preheated water and reagents and cooled with addition of cold water.

Detailed description of embodiments of the invention

The overflow drain of each dip tank may comprise a fluidics sensor, and the fluid control system may configured to individually interrupt the supply of fluid to one or several of the dip tanks upon the fluidics sensor's detection of the presence of fluid in the overflow drain. Accordingly, and easy-to-implement dosage system is provided, which ensures complete filling of the tank compartment without excessive spillage.

An automated microscope slide handling mechanism or robot may be provided for inserting microscope slides into the dip tanks and for removing microscope slides from the dip tanks. The slide handling mechanism or robot is preferably part of an automated staining apparatus, in which the system of the invention is integrated.

In order to fix a plurality of dip tanks at predetermined positions, e.g. within an automated staining apparatus, a dip tank holding structure may be provided for simultaneously holding an array of the dip tanks. With a view to enabling automated processing, an automated pipetting mechanism may be provided for pipetting a liquid into the dip tanks.

The agitator of the dip tank according to the invention may comprise a drive coupling structure for connecting the agitator to an external drive mechanism and for transmitting a driving force from the external drive mechanism to the agitator. The drive for driving the agitator within the dip tank may comprise a magnetic drive mechanism. Accordingly, the drive coupling structure may comprise a magnetic material, and the driving force may be a magnetic driving force. By the provision of a magnetic drive, the need for a mechanical drive interface is obviated, and a simple design of the drive interface between the drive and the agitator within the dip tank is provided, which requires a minimum of cleaning and maintenance.

The agitator may include a stirring bar, rod, wheel, screw, vane, spoon or any other element, including any propeller or mixer, capable of achieving a mixing effect within the liquid contained in the dip tank.

It should be understood that the reservoir of liquid, dosing pump, preheater, housing heater, fluid control system, reagent or buffer concentrate dispenser, waste reservoir and drive may be integrated with an immunohistochemical staining apparatus. In a preferred embodiment, the system of the invention comprises a single reservoir of liquid. In other embodiments, the

system comprises a plurality of reservoirs, each comprising a particular liquid, or a plurality of reservoirs, at least some of which comprise different liquids. In order to enhance mixing within the dip tank, the housing structure may be asymmetrical with respect to an upright axis extending between the upper and lower ends of the housing structure. Accordingly, the housing structure preferably has no rotational axis of symmetry.

The housing heater may comprise a heating foil wrapped around an outer surface of the housing, or alternatively or additionally a heating element, such as a heating coil, within the tank compartment for making direct contact with the liquid in the housing.

A temperature sensor is preferably provided for measuring the temperature of the fluid within the dip tank and for providing a control feedback to a temperature control unit of the system for controlling the housing heater.

Operation of a preferred embodiment of the invention is carried out as follows.

Warm water filling sequence:

The dip tanks are filled by the same dosing pump. When all dip tanks, of which there may be e.g. 12, are being filled and overflow sensors of the dip tanks are activated, and after a specified time, the pumping action is stopped. Then a common waste pump is activated for a few milliseconds, which reduces the filling level in all dip tanks. This action ensures both washing of slides and the filling of the tanks with warm water (about 65-70°C). Thereby the heat up time is reduced.

The dip tank liquid is heated to the operating temperature by the individual dip tanks' hosing heaters, such as heating coils, and kept there for the period specified by a target retrieval protocol, while being agitated. The specific target retrieval (TR) buffer concentrates (e.g. high or low pH) can be added by the dispenser prior to or after the processing temperature has been reached (typically about 98°C).

For the slides which do not require target retrieval with TR buffer at near boiling point as well as any dip tank without slide, the specific tank is not heated, and optionally no TR buffer concentrate is added. The slides stand passively in plain water or water containing e.g. detergents or pH stabilizing components, with slowly dropping temperature. Thereby the system can carry out individual target retrieval in an array of slides in dip tanks and even override target retrieval processes for individual slides.

Treatment of slides at above approximately 70°C is preferable in order to ensure target retrieval within a reasonable period of time.

In one embodiment of the invention, each dip tank can be provided with a dedicated filling and/or waste pump

Cooling down sequence:

The above filling process is basically repeated, however plain cold water is supplied from the dosing pump, while the liquid in the individual dip tanks is agitated but not heated.

The temperature will drop until below a certain limit (e.g. 40-45°C), from where the slides safely can be removed. Then the dip tanks are emptied and the slides removed.

Emptying:

All the dip tanks are emptied by activating the single waste pump for a specified period of time. Alternatively, a bottom valve can be opened and the liquid sent to the waste by pure gravity. A controllable waste pump is preferred, as this will assure better control and the system will not depend on the actual length of tubing or trapped air bubbles etc.

Further aspects and advantages of embodiments of the invention will now be described.

To obtain the best possible diagnosis in the shortest possible time the turn-around-time for an entire case comprising a plurality of microscope slides should be short, the target retrieval steps highly reproducible and the target retrieval protocols should preferably be chosen freely for each slide. In addition the instrumentation should be safe and easy to operate and be of a compact design which further does not require large quantities of heavy buffer consumables to be carried to the instrument.

Technical solutions to reduce the turn around time and improve treatment reproducibility and performance are associated with solving the major care-abouts and technical problems during the heat induced target retrieval of tissue:

Preferred features:

- Free choice of individual target retrieval procedures for each slide in the same case, incl. the possibility to override target retrieval for individual slides in the case

- Reproducible control of the target retrieval processing temperature, independent of the treatment of the other slides
- Uniform and high temperature during treatment
- Fast heating to the target retrieval temperature
- Fast cooling of the tissue before the next processing

Technical problems to be avoided:

- Evaporation leading to dry out of the tissue on the slide
- Low process temperature leading to long process time
- Slide-to-slide tissue or sample migration in shared baths (so-called "tissue floaters")
- Target retrieval buffer carry over between slides in the same case
- Carry over of target retrieval buffer to the next slide to be treated
- Accidental spills of the corrosive target retrieval buffer in the instrument
- Foaming of target retrieval buffers containing detergents
- High demands for instrument power supply during e.g. the heat up phase

The inventor has found that one efficient method of treating the tissue at a very precise temperature near the boiling point at atmospheric pressure and perfectly homogeneously across the slide and tissue is by immersing the slide into an agitated body of target retrieval buffer in a dip tank.

In order to obtain a reproducible agitation by simple means and a controllable temperature, the volume of target retrieval buffer should preferably be significant.

A larger body of target retrieval buffer with a large heat capacity will help to dampen any temperature fluctuations. By using a larger volume, the tissue is therefore protected from local hot spots during aggressive heating, which could cause damage to the morphology or

subsequent staining pattern. Also, any reduction of the volume due to evaporation is more easily controlled with a larger body of buffer.

In order to overcome the resulting problem of long heat up time, the inventor has realized that using pre-heated water in combination with a heating source at the dip tank will solve several technical drawbacks:

First, the warm up time is reduced, secondly, it averages the power consumption over time, especially when several target retrieval procedures are started at the same time, and thirdly, by using pre-heated water below 80 °C, the target retrieval process only starts if the temperature is further raised by the heating source at the dip tank. This is important for procedures where target retrieval is not wanted for a particular slide.

Also, the inventor has realized that the most efficient method of subsequently lowering the temperature after the target retrieval procedure and at the same time protect the tissue from drying out is by continuously substituting the warm target retrieval buffer with colder water.

Further, the inventor has recognized that this method allows the system to perform an automated warm washing, which is an important step in the so-called 3-in-1 protocol combining dewaxing, rehydration and target retrieval.

After many iterations, the inventor has designed a dip tank and fluidics system, which combines all the above features and at the same time is easy to incorporate into a larger instrument system and has several built-in safety features to ensure reproducibility in performance, reliability in operation and low maintenance and manufacturing cost.

Finally, the inventor has designed a dip tank with a trapezoid shape, so that when placed in an array, the tanks have a minimum of physical contact to reduce temperature cross talk. There is even space for insulation material between the dip tanks, despite a distance of less than 10 mm between the slides in neighboring tanks.

The prior art teaches away from using a heated dip tank arrangement for individual treatment of slides. This is likely due to the fundamental problems of long heat up and cool down times and difficulties in designing a practical arrangement with multiple dip tanks.

The prior art unanimously teaches that individual slide target retrieval in stainers is done with a small volume of buffer and with direct heating of the slide. The prior art stainer technology is consequently focused on covering the tissue and slide and protecting the small volume from the rapid evaporation near the boiling point of water.

The preferred target retrieval dip tank and system may comprise a number of design elements, including:

- Small tanks with a low volume for individual slides
- Mixer with an asymmetric mixing pattern
- Cold/warm water inlet
- Splash guard at top of water inlet
- Cold lid over tank
- Target retrieval buffer concentrate inlet in lid
- Large overflow drain
- Bottom drain controlled by pump
- Temperature sensor
- Fluidics sensor in overflow drain
- Asymmetric tank shape with minimal temperature cross talk between adjacent dip tanks
- Heating foil wrapped around individual tanks
- Pre-heating water tank
- Dosing pump for moving cold and pre-heated water to the dip tank
- 3 way valve for controlling inlet of cold or pre-heated water
- Waste pump
- In-line water purification device

- Target retrieval concentrate buffer delivery system for individual tanks

An embodiment of an antigen retrieval dip tanks is illustrated in figure 1 as seen from the side and above. The slide (1.1) is placed vertically in the middle and each tank has an inlet (1.2) for pre-heated and cold water, an overflow drain (1.3) with an overflow sensor, a bottom drain (1.4) controlled by a valve, a magnetic stirring bar (1.5) controlled by an external DC motor and magnet, a heating foil around the dip tank (not shown) and a temperature sensor (1.6). By this configuration, the slides can be both cold and warm washed and target retrieved according to the protocol for the individual slide. Also, a short heat-up and cool-down time is possible due to the use of pre-heated water from a temperature controlled tank and cold water directly from the internal water purification system.

As used herein, the term dip tank is a general term used for a device holding, mixing and controlling the treatment fluid. The dip tank is a generic term for all the designs covered by this invention.

The preferred dip tank materials include coated aluminum, stainless steel, ceramics, polymers like polypropylenes, polyethylene, poly amides, poly carbonates, silicones or nylons or similar industrial materials which are chemically resistant against the harsh target retrieval reagents.

Preferred embodiments of the invention result in several advantages compared to prior art, including:

- A dip tank for each individual slide
  Free choice of TR protocol for each slide in a case

- Individual agitation
  Fast mixing of concentrates and water
  Fast heating
  Uniform temperature distribution
  Fast cooling

- Simple fluidics and controls
  Cheap and safe with built-in controls to detect and correct errors or abnormalities

- Pre-heated water
  Faster heat-up time
  Low peak power demands

- TR procedure can be overridden for individual slides mounted on the same rack
  The slides are merely washed with pre-heated water but never subjected to the higher temperature where heat induced target retrieval proceeds

- "3in1" dewax/rehydration/target retrieval procedure is possible
  A specific dewaxing and rehydration process can be carried out prior to or after the target retrieval process;
  Water immiscible components, like the embedding paraffins, clearing agents or fatty components from the sample tissue are removed by the pre-heated water and by the flushing system.

It should be understood that the present invention is suited to both wash and target retrieve tissues mounted on slides.

In the following, examples of typical unit operations are described:

Full Target Retrieval:

1. Starting conditions at room temperature (e.g. 23 °C). The slide is placed in the dip tank

2. Heating I: Pre-heated water (e.g. 80-90 °C) from external tank is flushing into the dip tank, while stirring, until the tank temperature reaches a set temperature (e.g. 50-75 °C). Excess water is running out of the overflow drain.

3. Heating II: The inlet flow is stopped, the level adjusted by opening the waste drain pump (e.g. 200 milliseconds). Heating is continued only from the heating foil, until the desired process temperature, for example 98 °C

4. Addition of concentrated TR buffer, while stirring

5. Incubation phase with constant temperature at 98 °C for more than 20 minutes controlled by the heating tape

6. Cooling down (and washing) by flushing with cold water from the inlet, until the temperature is below 40-50 °C

7. Stopping the stirring, with microscope slide still in cold water. Slide ready to be moved to staining module

8. Slides are removed for the next process steps

9. Tank is emptied by pumping out from the bottom drainage

Extra washing of tank:

1. Flushing with cold water while stirring

2. The inlet flow is stopped when the outlet sensor is triggered or after a set time period. The level is adjusted by opening the drain pump for a short period

3. The stirring is stopped and the tank is emptied by pumping out from the bottom drainage

Ignoring target retrieval and doing dewaxing and rehydration:

1. Starting conditions at 23 °C

2. Heating I: Pre-heated water is flushing into the dip tank, while stirring

3. The inlet flow is stopped after a set time period, the level adjusted, no further heating

4. Passive "incubation phase" with temperature slowly decreasing with no stirring

5. Active cooling down (and washing) by flushing with cold water until temperature is below 40-45 °C.

6. Stopping the stirring, with microscope slide still in cold water. Slide ready to be moved to staining module.

7. Slides are removed for the next process steps

8. Tank is emptied by pumping out from the bottom drainage

It should be understood that the current dip tank arrangement allows for multiple combinations of washing and reagent treatment protocols, including combined enzymatic and heat induced antigen retrieval protocols and repeated heat induced antigen retrieval with e.g. high and low pH buffers.

Preferred designs in detail:

The dip tank may have a sloped bottom for easy drainage. This will make it easier to empty, reduce carry over and increase the washing efficiency.

The preferred dip tank design includes a lid covering most of the dip tank. An even more preferred design includes a so-called cold lid, which is not heat isolated and allows heat to actively or passively escape. Thereby the vapors from the dip tank will condensate on the colder underside of the lid and drip back into the dip tank. This reflux action will further increase the temperature stability of the system near the boiling point.

In order to enhance the reflux capability, the lid can be connected to a cooling device or even to a dedicated reflux column, as is well-known from the organic chemistry laboratory.

In the preferred array of dip tanks the tanks share the same dosing pump and waste pump. This is a cost effective design.

The preferred dip tank arrangement also has several built-in safety mechanisms.

For example, by monitoring the temperature versus time for each dip tank, any abnormalities can be detected for the individual dip tank. If the heating foil is not working or the dip tank not filled, the temperature will not raise in a normal way and the control software can make corrective actions or simple shut down the particular dip tank and allow the other dip tanks to operate.

Another example of a detectable error could be blocked or unconnected fluidics lines or dosing or waste pump malfunctions, which can be detected by the signal from the fluidics sensor in the overflow drain. This combined with appropriate allowed time delays could detect errors in specific dip tanks, which could then be excluded and identified for repair.

As the target retrieval buffers are highly corrosive, the above described safety features are much appreciated in a complicated automated instrument with numerous electric boards and wire connections. Also, errors can be narrowed down to single dip tanks, allowing the other tanks to be operated and thus maintain the stainer's productivity, though at a lower level.

The mixing can be of the paddle type with an overhead shaft or more preferably a magnetic stirrer with a small magnetic mixer bar and a permanent magnet outside the dip tank. Preferably, the mixing bar is permanently held in position in the dip tank to avoid tumbling or loss of the stirring bar.

Preferably, the mixing bar is placed asymmetrically in the tank to promote an efficient mixing and agitation process. It is preferred that the agitation is enough to homogenize the solution within seconds and not so strong that tissue mounted on the slide is dislodged or foam is built up when using buffers containing detergents.

Alternative designs and uses:

Another preferred heating and agitation method is by pumping the buffer in and out of the dip tank and preferably across an in-line heating device. Thereby, the heating and mixing is very efficient, the system can be miniaturized, the dead volume, the heat up and cool down time reduced.

It should be understood that the present dip tank and arrangement is also well-suited for other treatment procedures involving samples mounted on slides.

Examples include primary staining using Hematoxylin and erosin and special staining procedures. Not only the special stains which are done at room temperature but also special stains requiring elevated temperature can be carried out in the dip tank, including various aluminum Hematoxylin stains, various Giemsa stains, Grocott´s methenamine silver staining, periodic acid shiff (PAS), acid fast, Stainer-Stainer, Warthin-Starry, Mayer Mucicarmine, Dieterle, Helicobacter, Congo Red, Sudan Black, Jones, Feulgen, methyl green-pyronin Y, Urate Crystals, iron, Masson's Trichrome, Gomori's Trichrome, Bielschowsky, acid-fast bacillus (AFB) and Gram staining procedures.

In addition cytological staining procedures, like PAP staining, may be conducted in the dip tank of the invention.

Furthermore advanced stains like immunological stains using antibodies (IHC) can be done in the dip tank. Due to the capability to do procedures at elevated temperatures even staining procedures using molecular probes, in-situ hybridization can be performed.

Description of the drawings

Embodiments of the invention will now be further described with reference to the accompanying drawings, in which:

Figure 1 are schematic drawings of one embodiment of a target retrieval dip tank with an inserted slide as seen from the side and above. The low volume tank includes a combined fresh and preheated water inlet, overflow and bottom drainage, stirring bar and temperature sensor.

Figure 2 is a schematic drawing of the fully automatic test set-up fixture, including dip tank, slide, DC motor with permanent magnets, dosing and waste pumps, reservoir (7) for preheating fresh water, a standard circular robot and dispensing pipette.

Figure 3 is a general flow and control scheme for the fully automatic test set-up fixture, including the dip tank and overflow sensor, pre-heated fresh water reservoir with heater and sensors, dosing pump, fresh water pump, cold water reservoir, 3-way switch and waste pump.

Figure 4 is the general procedure scheme used during the temperature ramp up and cool down procedure.

Figure 5 is a graph illustrating the full target retrieval procedure temperature profile during heat up and cool down. The external temperature is measured on the slide and the internal temperature at the bottom of the dip tank.

Figure 6 is a simplified line drawing of an embodiment of an automated staining apparatus.

Figure 7 illustrates the dewaxing/rehydration and the target retrieval modules with the shared robot arm. The arm is mounted with dispensers capable of delivering dewaxing and rehydration reagents and target retrieval buffer concentrates. Also, the arm is equipped with an air knife for gentle removal of liquids on the slides placed in the dewaxing module. The fluidics and air tubing are not made visible.

Figure 8 is a schematic drawing of the dip tanks assembly in the target retrieval module, illustrating the array of tanks and the low inter tank contact surface. Also, the drawing shows the resting position and wash station for the robotic buffer dispensers.

Figures 9, 10 and 11 are line drawings of an embodiment of the staining module with mixing grid and reagent delivery probe in an automated staining apparatus. More specifically, figure 9 illustrates the slides in the slide racks being lowered down into the staining module by the overhead gantry robot. Figure 10 illustrates the combined x-y-z reagent probe and air knife robot arm capable of sip-and-spit delivering of the specific reagents from the reagent bay to the slide and grid assembly, and air knife cleaning of the mixing grids. The fluidics lines are not illustrated. Figure 11 illustrates the slides and grids assembled as during reagent dispensing, incubation and washing, with the reagent probe positioned over the drop channel of one of the grids.

Figure 12 shows the slides and grids separated and the air knife positioned to clean the grids.

Figure 13 is a drawing of an example of an embodiment of an inset for holding bottles and containers in the reagent drawer. Note the polarity of the inset, which prevents wrong placement and positioning of the reagent bottles.

Figure 14 includes several schematic drawings of examples of embodiments of reagent bottles and insets for the reagent drawers.

Figures 15-17 are simplified sketches of embodiments of an automated staining apparatus.

Example 1 - Preparation of target retrieval dip tank prototype and testing set up

An example of an individual target retrieval dip tank was prepared in printed polyamide (similar to PA6) as a rapid prototype by selective laser sintering (SLS) (ModellTechnik Rapid Prototyping GmbH, Germany) according to the e-drawing (Solid Works) illustrated in figure #1. The Nylon was treated with a clear varnish to protect the surface.

As seen in figure 1, the slide (1.1) is placed vertically in the middle and each tank has an inlet (1.2) for pre-heated and cold water, an overflow drain (1.3) with an overflow sensor, a bottom drain (1.4) controlled by an valve/pump, a magnetic stirring bar (1.5) controlled by an external DC motor and magnet, a heating foil around the dip tank (not shown) and a temperature sensor (1.6).

A fully automatic test set-up fixture was built for evaluating the dip tank's performance, including fluidics handling and temperature.

Figure 2 is a schematic drawing of the test set-up fixture built on a standard breadboard basis (Thorlabs, BIT Analytical Instruments, Schwalbach, Germany).

In short, the standard (Menzel) microscope slide (2.1) was placed in the slit in the lid (2.2) and partly immersed in the dip tank (2.3). The magnetic stirring bar was placed in the dip tank. Below the dip tank, the DC motor (2.4) (cat no. SFF-030VAV, SGST) with permanent magnets was mounted on a supporting plate (1.5 mm thickness; BS EN 1.4301 stainless steel sheet), together with a dosing pump for delivering warm/cold fresh water to the dip tank and a waste pump (2.6) for emptying the dip tank.

Next to the dip tank assembly a reservoir (2.7) for preheating of fresh water was placed with level sensor, electric heater and thermometer sensor.

Also, a standard circular robot (2.8) (Theta-Z Robotic Arm, cat. no 71905220) and dispensing pipette (2.9) (inner/outer diameter 0.6/1.0 mm) (both from BIT Analytical Instruments), were mounted next to the dip tank for automatic dispensing of liquids directly into the dip tank during tests.

The dip tank was mounted with a thermo sensor (Betatherm NTC thermistor) in the bottom, a self calibrating fluidic sensor at the overflow drain.

The preheated fresh water reservoir, dosing pump (Micro diaphram pump, up to 100 ml/min, NF10 KPDC, KNF), cold water reservoir and a 3-way valve switch (3/2 Valve cat. No. FAS F09055 20-09 from Bürkert) and waste pump were connected with tubing (standard Tygon) as described in the general flow scheme in figure #3.

Further, the dip tank was wrapped with a heating foil (Betatherm, 12V/48W) and additional isolation material to minimize heat loss. The entire test set-up was remotely controlled by a standard general module board, software and a simple user interface (FingerTip Version 3.2 Build 2, all from BIT Analytical Instruments).

Various procedures for dilution, mixing, heating up, cooling down and washing with warm or cold water could be tested with the test set.

In the following examples, various dip tank properties were recorded using the automatic set-up. Where possible, the performance was recorded with a still and video camera (5 MP CSOS digital camera).

All electronic input and output were collected for post analysis, including static analysis.

Example 2 - The temperature ramp up time, temperature stability and ramp down time

In this example, preheated water was pumped into the heated dip tank for a period to reduce the total heating up time before the heating alone heated up the dip tank. Also, during cool down, cold water was pumped into the dip tank to fast reduce the temperature and wash the tank.

Figure 4 depicts a specific procedure scheme used during the temperature ramp up and cool down procedure.

In short, a) the preheated water reservoir was filled, b) fresh water pump started, c) fill level sensor activated, d) fresh water pump stopped, c) heating of preheated reservoir started, d) temperature sensor at 95 °C, e) heating of preheated reservoir stopped, f) 3 way valve switched to open to preheated water reservoir, g) dosing pump started, h) mixer started, i) temperature measurement started, j) heating in dip tank (foil) started, k) overflow sensor activated, l) dosing pump stopped after 30 seconds, m) waste pump started for 200 milliseconds, n) dip tank heating to 98.5 °C, on/off according to control algorithm, o) incubation for 25 minutes, p) 3 way valve switched to open to cold water reservoir, q) dosing pump started, r) overflow sensor activated, a) dosing pump stopped after 60 seconds, t) waste pump started for 200 milliseconds, u) stirring stopped, x) heaters turned off.

Figure 5 is a graph illustrating the full target retrieval procedure temperature profile. The external temperature was measured with a sensor on the slide surface and the internal temperature at the bottom of the dip tank. The temperature curves are parallel.

The external temperature was verified against a standard. The internal temperature was with an uncorrected offset.

As illustrated in the graph, the TR procedure is easily followed by the temperature curve. The process goes through 6 phases:

1. Starting conditions at 23 °C

2. Heating I: Preheated water was flushing into the dip tank, while stirring, until 63 °C
3. Heating II: The inlet flow stopped, the level adjusted, only heating from the heating foil, until 98 °C
4. Incubation phase with constant temperature at 98 °C for more than 20 minutes
5. Cooling down (and washing) by flushing with cold water, until temperature below 45 °C
6. Stopped, with microscope slide still in cold water and with no stirring. Slide ready to be moved to staining module.

By analyzing the recorded data, the average temperature over 25 minutes was calculated to 98.5 °C with a standard deviation of 0.22°C.

Also, using the cold-water-flush method, the temperature could be lowered from 98.5 °C to below 45 °C in less than 18 seconds.

In summary, using a warm-water-flush method, heating foil and stirring in the dip tank, the temperature could be raised from 23 °C to 98 °C in 232 seconds, or less than 4 minutes.

The process parameters, including preheating reservoir temperature, preheated water volume and temperature-time-power algorithm for controlling the heating foil, have been mapped for further optimization.

Also, an error correcting scheme has been constructed based on the feed back information from the temperature-time curve.

Example 3 - Mixing and washing efficiency

The mixing efficiency was estimated using dyes in the same general set-up as in example 2, except for no heating and without lid.

In short, a strongly colored dye solution was prepared by dissolving 30mg of Thymol Blue (thymolsulphonephthalein, cas no 76-61-9, Sigma-Aldrich, cat. No 114545-5G) in 50 ml demineralized water. 5 mg of NaOH pellets (Fluka, cat. No. 71691) were added to dissolve the Thymol Blue and homogenized by a vortex mixer (IKA: MS 3 digital) for 10 minutes.

The dip tank was filled with 24 ml demineralized water using the standard dosing pump method, the mixing was stopped and 100 µl dye solution dispensed by the automatic robot. The strong blue dye drops were clearly seen in the dip tank.

The mixing was started and the mixing pattern observed and a video recorded. The experiment was repeated both with slide and without slide.

Mixing was complete within 3 seconds, both with and without a slide in the dip tank. The liquid in the dip tank became light bluish without any visibly inhomogeneous areas.

After realizing the homogeneous mixture in the dip tank, the washing process efficiency was estimated.

The mixing was continued and the dosing pump was started and excess water ran into the overflow drain.

The dye was clearly washed out and the liquid was colorless with no traces of blue color against the white dip tank interior.

Without slide, a complete washing of the dip tank was realized within 30 seconds, according to the time sequence of the photos taken from the video sequence.

With a slide inserted in the dip tank, a complete washing of the dip tank was realized within 20 seconds or less. The inserted slide seems to increase the speed of substituting the colored water with clean colorless water. A closer study of the video sequence indicates a split stream mixing mechanism around the slide in addition to the efficient circular mixing movement from the mixing bar in the bottom.

In conclusion, diluting and mixing of a small dispensed liquid volume into the larger volume in the dip tank was completed within 3 seconds, Also, complete washing of the dip tank could be realized after less than 30 seconds.

Example no 4 - Carry-over measurements

The carry-over was quantified in experiments using a typical protocol for change of buffer. As the pH is the most critical parameter in the target retrieval procedure, the carry-over was quantitatively measured as the change in pH when changing the type of target retrieval buffer system in the dip tank.

The general set-up was the same as described in example no 2, except that no heating or cooling protocol steps were included in this experiment, all in order to limit any uncontrolled

effects from the special prototype polyamid material and potential diffusion of carbon dioxide to and from the atmosphere.

Two different target retrieval buffer concentrates were used:

250 µl low pH target retrieval buffer concentrate (PT Module Buffer 1 Thermo Scientific cat. no TA-125-PM1X; 100x citrate buffer, pH=6), and 250 µl high pH target retrieval buffer concentrate (PT Module Buffer 4, Thermo Scientific cat no. TA-125-PM4X; 100 mM Tris/1 mM EDTA, 100x citrate buffer, pH=9).

During the experiment, the concentrates were diluted in demineralized water in the 24 ml dip tank volume, according to the recommendation by the manufacturer.

A calibrated pH-meter (S20 SevenEasy, Mettler Toledo) was used to perform the pH measurements.

The testing cycle was the following:

First the fresh water reservoir was filled, b) fresh water pump started, c) fill level sensor activated, d) fresh water pump stopped, c) valve switched open to water reservoir. Subsequently, the dip tank was operated: d) dosing pump started, e) overflow sensor activated, f) dosing pump stopped, g) waste pump started for 200 milliseconds, h) 250 µl high target retrieval buffer concentrate added by the pipette, I) mixer started, j) after incubation for 20 minutes, pH was measured, k) dosing pump started for 30 seconds, m) waste pump started for 20 seconds and n) stirring stopped.

This cycle was repeated first with high pH TR buffer, three times with low pH TR buffer and finally with the high pH TR buffer.

The table below summarizes the cycles and the pH measurement.

Cycle no	Buffer #1, pH units	Buffer #2, pH units	Change in pH
#1 – pH normal		8.28	
#2 – pH carry over	6.67		

#3 – pH nominal	6.56		
#4 – pH nominal	6.54		(#4-#2): +0.13
#5 –pH carry-over		8.30	(#5-#1): +0.02

The above testing procedure was a worst case scenario, as the TR buffer was changed from high to low and back to high pH again and no separate washing procedure introduced. Also, the surface of the nylon rapid prototype dip tank was rough and had not been polished.

In conclusion, the pH carry-over was less than 0.13 (from high to low: 0.13 and low to high: 0.02), which is less than what is recommended by the TR buffer manufacturer (0.15) as the maximum deviation when making the TR buffer directly.

Example no 5 - Test of foaming

Foaming of reagents during the target retrieval procedure is a potential disturbing phenomenon which could block liquid sensors or cause staining artifacts.

The TR buffers both contain detergents and mixing could potentially drag air into the solution or promote foaming.

The test set-up was as in example no. 2 and 5. Four different experiments were conducted for quantifying foaming phenomena during constant mixing: Using the high pH TR (pH 9) buffer for 20 minutes at room temperature and at 98,5°C and the low pH TR buffer (pH 6) for 20 minutes at room temperature and at 98,5°C.

The dip tank was observed and a video recorded. When dispensing of the concentrate, a few bubbles were observed, but they vanished within seconds of mixing.

No persistent foam was observed after 20 minutes at 98,5°C and at full speed mixing using either the low pH citrate or high pH TRIS TR buffers

In conclusion, no foaming was observed in the worst case experiment combining vigorous mixing, heating and using TR buffers known to easily form foam.

Example no 6 - Measurement of evaporation

The degree of evaporation was quantified in an experimental set-up similar to example no. 2 and 5 with vigorous mixing for 20 minutes and at 98.5 °C. The experiment was done with lid – but without a slide inserted in the dip tank.

After 20 minutes, the liquid was removed and weighted. Less than 1.5 ml out of the 24 ml in the dip tank was evaporated.

As a control experiment, the same experiment was done without the lid and slide. About 5-6 ml of the liquid had evaporated after 20 minutes.

In conclusion, without lid, evaporation was significant, indicating that the lid has a significant impact on the reflux of condensed water into the dip tank.

With the lid mounted, no significant evaporation was observed in the experiment at near boiling for 20 minutes and with no slide in the slit in the lid. The cold lid reflux design worked.

End of example 6.

As schematically illustrated in figure 6, an embodiment of an automated staining apparatus comprises several treatment modules and robots, including a drawer (6.1) for loading and off loading racks, an overhead gantry robot (6.2) that can grab, lift, transport, lower and release slide racks into the various positions in the apparatus, a storage room (6.3) for multiple slide racks, a warm air baking and drying module (6.4) harboring more than one slide rack, a dewaxing and rehydration module (6.5), a target retrieval module (6.6) with an array of target retrieval dip tanks, a staining module (6.7) with mixing grid, an overhead x-y-z reagent delivery robot (6.8) with a multidispensing reagent probe and air knife, a reagent bay or module (6.9) harboring multiple specific reagent containers under temperature control and accessible for loading and changing through separate drawers (6.10).

The lower part of the staining apparatus comprises a number of bulk reagent containers (6.11) for wash and target retrieval buffer concentrates, dewaxing, rehydration and dehydration solutions, in addition to waste containers for organic (6.12) and toxic aqueous

waste (6.13) and an internal water purification module (6.14) capable of purifying tap water for use in the apparatus. The apparatus has a connection to the general sewage system for the non-toxic aqueous waste. The apparatus has a supporting and stable frame (6.15) mounted with wheels (6.16).

The apparatus can store and process several slide racks at the same time. The gantry robot moves the racks between the treatment, storage and loading modules.

In the following, some of the technical functionalities in an embodiment of an automated staining apparatus is described in more detail.

Also, in figures 7 through 14 the functionalities of the apparatus is illustrated in more detail.

The loading drawer can be opened to allow a slide rack to be inserted or removed. When the drawer is automatically closed, labels on the slide and racks are read by a reader positioned in the apparatus. When the drawer is closed, the gantry robot can pick up the rack and move it to any of the other modules in the apparatus.

The storage compartment has space for multiple racks placed in a vertical position. The environment with respect to humidity and temperature can be controlled.

The baking and drying module can harbor two racks in the longitudinal compartment. Air is taken in by a fan, heated by a heating coil (40-60 $^{\circ}$C) and pushed past the slides in the rack before being exhausted. Any aqueous liquids are removed by a small drainage in the bottom and melted embedding media (e.g. paraffin) is collected by a hydrophobic pad. After treatment, the rack is moved to the next position by the gantry robot.

The dewaxing/rehydration module and target retrieval module are illustrated in figure 7.

The slide rack is lowered into the dewaxing rehydration module with the slides in a near vertical position. The rack is inserted into a locking mechanism, released from the gantry robot and the rack turned into near horizontal position with a small 2-5° inclination.

As illustrated in figure 7, the dewaxing/rehydration module and target retrieval module share a robot rail (7.1) and reagent delivery robot arm (7.2). Dewaxing, rehydration or optional dehydration reagent is heated by an in-line heater (7.3) and dispensed according to the protocol to each of the slides through separate probes (7.4).

After an incubation time, the liquid is removed from the slide surface by the air knife (7.5) also on the robot arm. After treatment, the rack holding the slides (7.6) is again turned to vertical position and moved to the next position by the gantry robot.

In the target retrieval module, the slide rack is lowered down by the gantry robot and each slide inserted into a slit in the lid (7.7) and into small individual target retrieval dip tanks below. The lid also has small holes over each dip tank through which the robot arm's target retrieval probe (7.8) can deliver one of three different buffer concentrates. The dispensing probes are controlled in the z direction by the motor (7.9) and x direction by the rail (7.1).

The individual retrieval dip tanks are illustrated in figure 1 as seen from the side and above. The slide (1.1) is placed vertically in the middle and each tank has an inlet (1.2) for pre-heated and cold water, an overflow drain (1.3) with an overflow sensor, a bottom drain (1.4) controlled by an valve, a magnetic stirring bar (1.5) controlled by an external DC motor and magnet, a heating foil around the dip tank (not shown) and a temperature sensor (1.6). By this configuration, the slides can be both cold and warm washed and target retrieved according to the protocol for the individual slide. Also, a short heat-up and cool-down time is possible due to the use of preheated water from a temperature controlled tank and cold water directly from the internal water purification system.

A plurality of target retrieval dip tanks of the type depicted in figure 1 are placed in an array as illustrated in figure 8. The tanks (8.1) are placed close together, but also have a minimum of physical contact due to the trapezoid shape. An isolation material between the tanks prevents temperature cross talk. The array also includes a resting position and washing station (8.2) for the target retrieval probes. After treatment, the slide rack is moved to the next treatment module by the gantry robot.

The gantry robot and the staining module is illustrated in figure 9.

The overhead gantry robot (9.1) lowers the slide rack (9.2) into the locking mechanism (9.3) in the staining module. The staining module contains 12 mixing grids mounted with springs on a common rail. Each of the grids has a drop channel (9.4) and the grids can be moved transversally back and forth by the motor (9.5).

The drop channels of all the grids are positioned in the same general plane as the covering plate over the reagent bay to allow the reagent robot to easily take up reagents through the holes (9.6) and deliver them to individual drop channels in the staining module.

The reagent probe and air knife robot are illustrated in figure 10.

The multidispensing reagent probe (10.1) is equipped with a crash detector and can sip reagents from any of the reagent containers in the bay and deliver to any of the grids and slides assemblies. The air knife (10.2) is at a fixed angle on the robot arm. The x-y direction are controlled by the overhead rail system (10.3) and the z direction by a small rail and motor system (10.4).

During the staining procedures, the slide and grid are assembled. This is illustrated in figure 11.

During reagent dispensing, incubation and washing according to the staining protocol, the slide rack (11.1) is turned to a near horizontal position with a slight angle of 0-5°.

The individual grid rests on the slide with a gap in between them. The reagents are delivered to the drop channels (11.2) by the reagent probe (11.3) from the reagent bay (11.4) or wash buffer container (not shown).

The reagent (about 100 microliters) is held between the slide and the grid during incubation, and when a larger wash buffer volume is added in the drop channel, the reagent is displaced by the wash buffer. The excess liquid is allowed to run over the edge of the slide during the washing step.

The grids are moved transversally back and forth to promote agitation of the reagents and speed up the diffusion and reactions during the staining and during the washing procedure.

After each washing sequence, the grids and slides are separated. This is illustrated in figure 12.

The slide rack is turned downwards to a vertical position (12.1) and the grids upwards (12.2). This allows the air knife (12.3) on the robot arm to clean each grid and at the same time allows any remaining reagents on the slide to passively run off and down into a waste pan (not shown). The slide and tissue is thereby isolated from the strong air stream.

By turning the grid assembly and slide rack, the grid can be cleaned and dried in between the slide staining and washing procedures. After staining and optional counter staining and dehydration, the slide rack is moved to the loading station or storage by the gantry robot.

The reagent bay holds the multiple reagents under temperature controlled conditions. During loading, change or emptying of the reagent bay, the machine readable labels are recorded by

a pair of readers on each side of the drawer. In one preferred design, the apparatus uses only two readers, which can be moved from drawer to drawer on a rail. When the specific drawer is closing, the reader can record the container labels, as the labels pass the two readers.

In one preferred embodiment, the reagent containers and bottles are placed in a reagent drawer inset.

Figure 13 shows an example of an inset for holding the specific reagent containers.

The drawer inset is polar, which prevents wrong placement and positioning of the reagent bottles. Further, as the apparatus uses numerous different sizes of bottles, in one preferred design, the apparatus has a set of bottles and insets which fits together.

For example, 50, 15, 5 and 2 ml bottles have the same height and opening mouth and placement of machine readable label. The polar insets allow the 15, 5 or 2 ml bottles to safely fit in the larger 50 ml bottle space in the reagent drawer for maximum loading and positioning flexibility. The smaller bottles can be inserted as single or double configuration in each inset.

This is illustrated in figure 14, with 50, 15, 5 and 2 ml bottles (14.1, 14.2, 14.3, 14.4) and the corresponding inset (14.5) for the 15 and 5 ml bottles (14.2, 14.3) to occupy the space of a 50 ml bottle and the inset (14.6) for one or two 2 ml bottles (14.4).

Figure 11, 12 and 13 illustrate a preferred staining apparatus embodiment skin, which gives easy access to a touch screen, drawer for slide rack, drawers for specific reagent containers, doors for access to bulk reagents and waste containers and a pull out table.

Figure 15 is a simplified sketch of an embodiment of an automated staining apparatus with a functional skin, including touch screen (15.1), drawer for slide rack (15.2), three drawers for specific reagent containers (15.3), doors for access to bulk reagents (15.4) and waste containers (15.5) and a pull out table (15.6).

Figure 16 is a simplified sketch of an embodiment of an automated staining apparatus with a functional skin seen from the front and side, including a 170 cm tall reference person, including touch screen (16.1), pulled out drawer for slide rack (16.2) and specific reagents.

Figure 17 is a sketch of an embodiment of an automated staining apparatus with a functional skin. To the left with open door to the bulk reagents (17.1) and to the right with the top lid open for access to the robotics during repair and service (17.2).

5

CLAIMS

1. A system for washing and target retrieval of tissue samples mounted on microscope slides, comprising a plurality of dip tanks, each of which comprises:
- a housing structure and, within the housing structure, a holding structure for holding a single one of the microscope slides in a fixed position relative to the housing structure; the housing structure defining a tank compartment having an upper open end for receiving the microscope slide, and a lower essentially closed end opposite to the open end;
- a fluid inlet and a fluid outlet;
- an agitator for agitating fluid in the tank compartment;
said system further comprising:
- a reservoir of liquid;
- a dosing pump for pumping a controlled dosage of liquid from the reservoir to the fluid inlet of each of the dip tanks;
- a preheater for heating liquid pumped from the reservoir to the dip tanks;
- a fluid control system configured to allow liquid pumped from the reservoir to the dip tanks to selectively bypass the preheater;
- a housing heater for heating at least a portion of the housing of each of the dip tanks;
- an overflow drain associated with each one of the dip tanks;
- a drive for driving the agitator within the dip tank;
- a waste reservoir for liquid;
- at least one conduit connecting the fluid outlet of the dip tanks to the waste reservoir.

2. A system according to claim 1, wherein the overflow drain comprises a fluidics sensor, and wherein the fluid control system is configured to individually interrupt the supply of fluid to each of the dip tanks upon the fluidics sensor's detection of the presence of fluid in the overflow drain.

3. A system according to claim 1 or 2, further comprising an automated microscope slide handling mechanism for inserting microscope slides into the dip tanks and for removing microscope slides from the dip tanks.

4. A system according to any of claims 1-3, further comprising a dip tank holding structure for simultaneously holding an array of said dip tanks.

5. A system according to any of claims 1-4, further comprising an automated pipetting mechanism for pipetting a liquid into the dip tanks.

6. A system according to any of claims 1-5, wherein said drive for driving the agitator within the dip tank comprises a magnetic drive mechanism.

7. A system according to any of claims 1-6, wherein said reservoir of liquid, dosing pump, preheater, housing heater, fluid control system, waste reservoir and drive are integrated with an immunohistochemical staining apparatus.

8. A dip tank for washing and target retrieval of a tissue sample mounted on a microscope slide, comprising:
- a housing structure and, within the housing structure, a holding structure for holding a single microscope slide in a fixed position relative to the housing structure; the housing structure defining a tank compartment having an upper open end for receiving the microscope slide, and a lower essentially closed end opposite to the open end;
- a fluid inlet and a fluid outlet;
- a housing heater for heating at least a portion of the housing structure;
- an agitator for agitating fluid in the tank compartment.

9. A dip tank according to claim 8, further comprising an overflow drain at or near the upper end of the housing structure.

10. A dip tank according to claim 8 or 9, wherein the agitator comprises a drive coupling structure for connecting the agitator to an external drive mechanism and for transmitting a driving force from the external drive mechanism to the agitator.

11. A dip tank according to claim 10, wherein the drive coupling structure comprises a magnetic material, and wherein the driving force is a magnetic driving force.

12. A dip tank according to any of claims 8-11, wherein the agitator is a stirring bar.

13. A dip tank according to any of claims 8-12, wherein the housing structure is asymmetrical with respect to an upright axis extending between the upper and lower ends of the housing structure.

14. A dip tank according to any of claims 8-13, wherein the housing heater comprises a heating foil wrapped around an outer surface of the housing.

15. A dip tank according to any of the preceding claims, further comprising a temperature sensor for measuring the temperature of the fluid within the dip tank.

16. A method for washing and target retrieval of tissue samples mounted on microscope slides, comprising a plurality of dip tanks, each of which comprises:

- a housing structure and, within the housing structure, a holding structure for holding a single one of the microscope slides in a fixed position relative to the housing structure; the housing structure defining a tank compartment having an upper open end for receiving the microscope slide, and a lower essentially closed end opposite to the open end;
- a fluid inlet and a fluid outlet;
- an agitator for agitating fluid in the tank compartment;

said method comprising:

- supplying a controlled dosage of liquid from a reservoir of liquid to the fluid inlet of each of the dip tanks;
- controlling the supply of liquid to allow liquid pumped from the reservoir to the dip tanks to selectively pass through a preheater for heating the liquid, and to allow liquid pumped from the reservoir to the dip tanks to selectively bypass the preheater;
- heating at least a portion of the housing of at least some of the dip tanks;
- agitating the liquid in the dip tanks;
- conveying the fluid from the dip tanks to a waste reservoir.

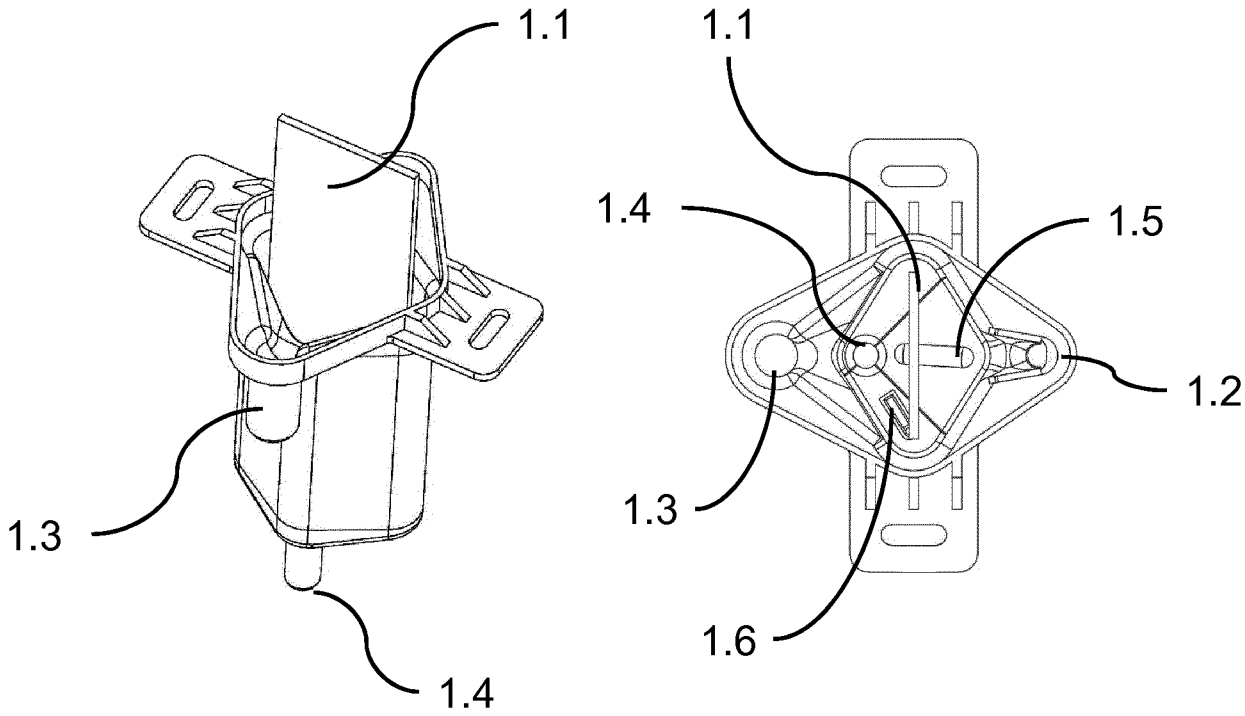

Figure 1

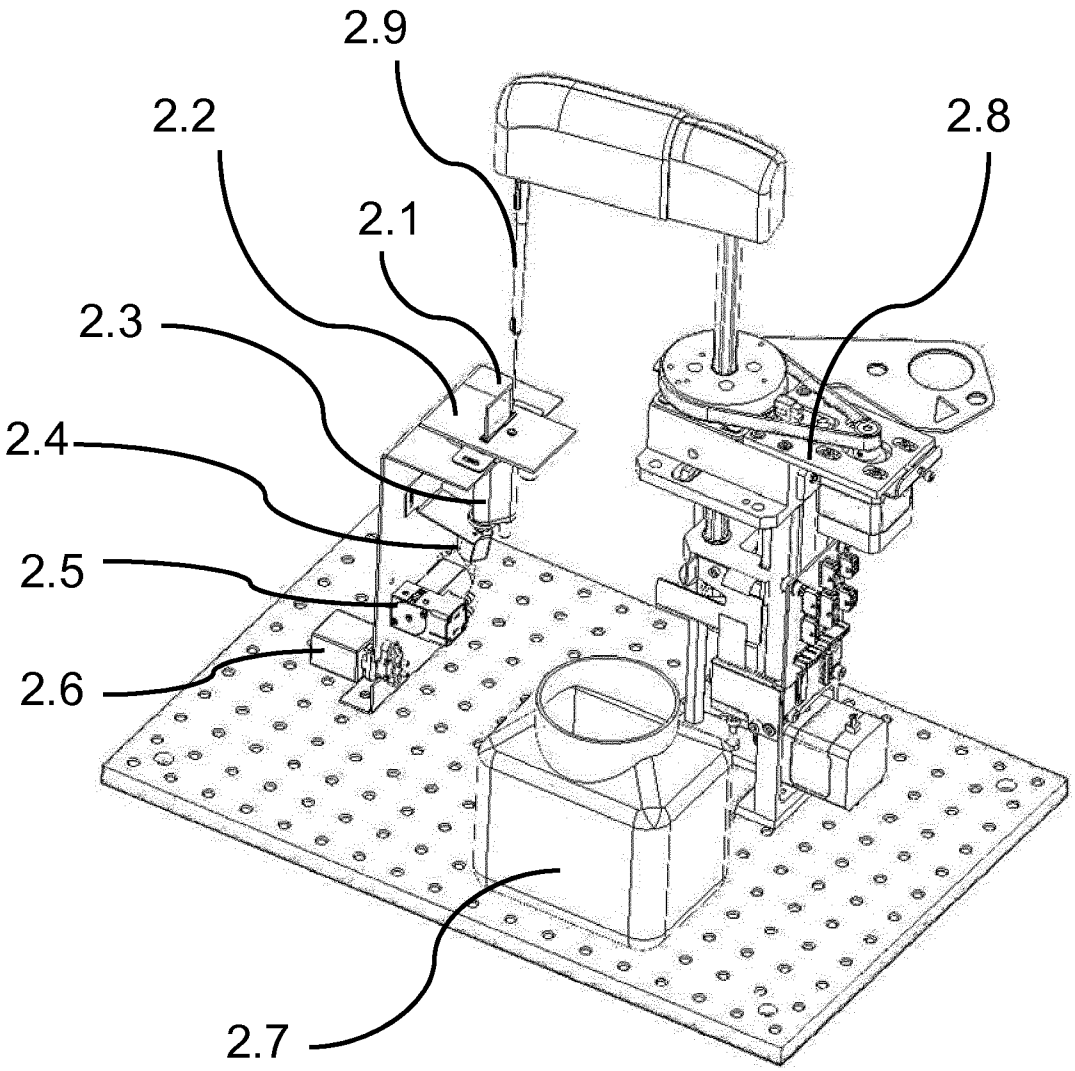

Figure 2

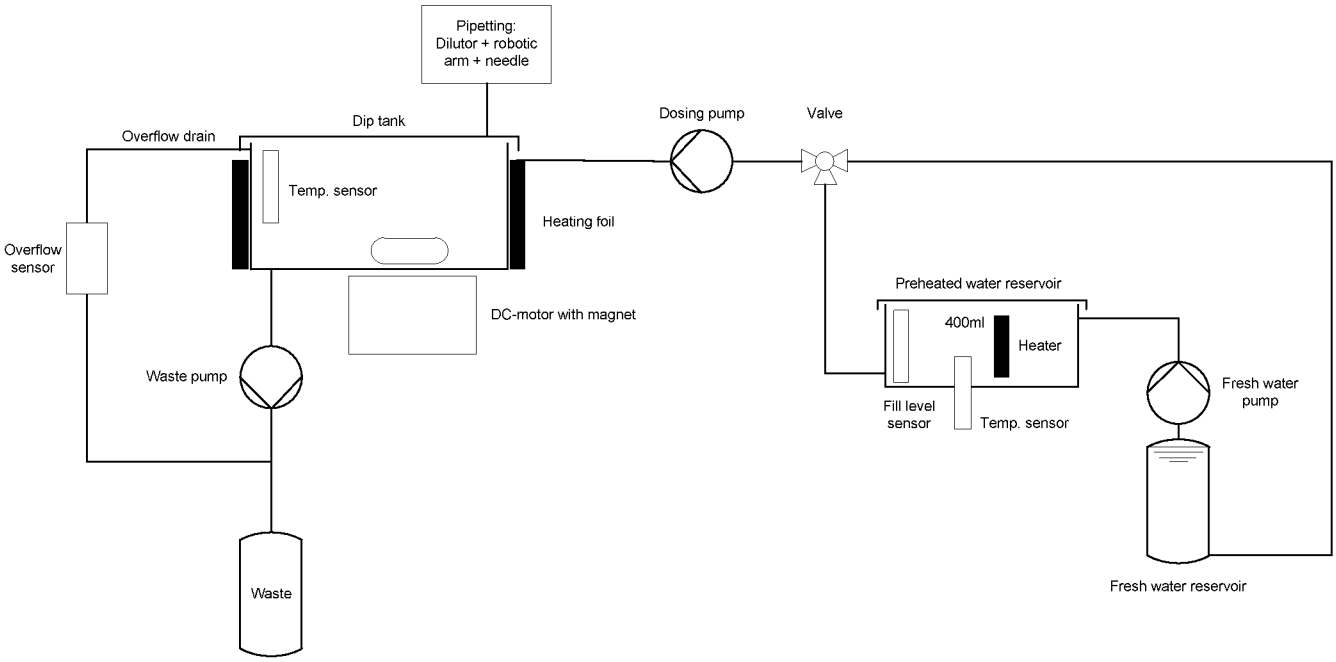
Pipetting:
Dilutor + robotic
arm + needle
Overflow drain
Dip tank
Dosing pump
Valve
Temp. sensor
Heating foil
Overflow
sensor
DC-motor with magnet
Preheated water reservoir
400ml
Heater
Waste pump
Fill level
sensor
Temp. sensor
Fresh water
pump
Waste
Fresh water reservoir

Figure 3

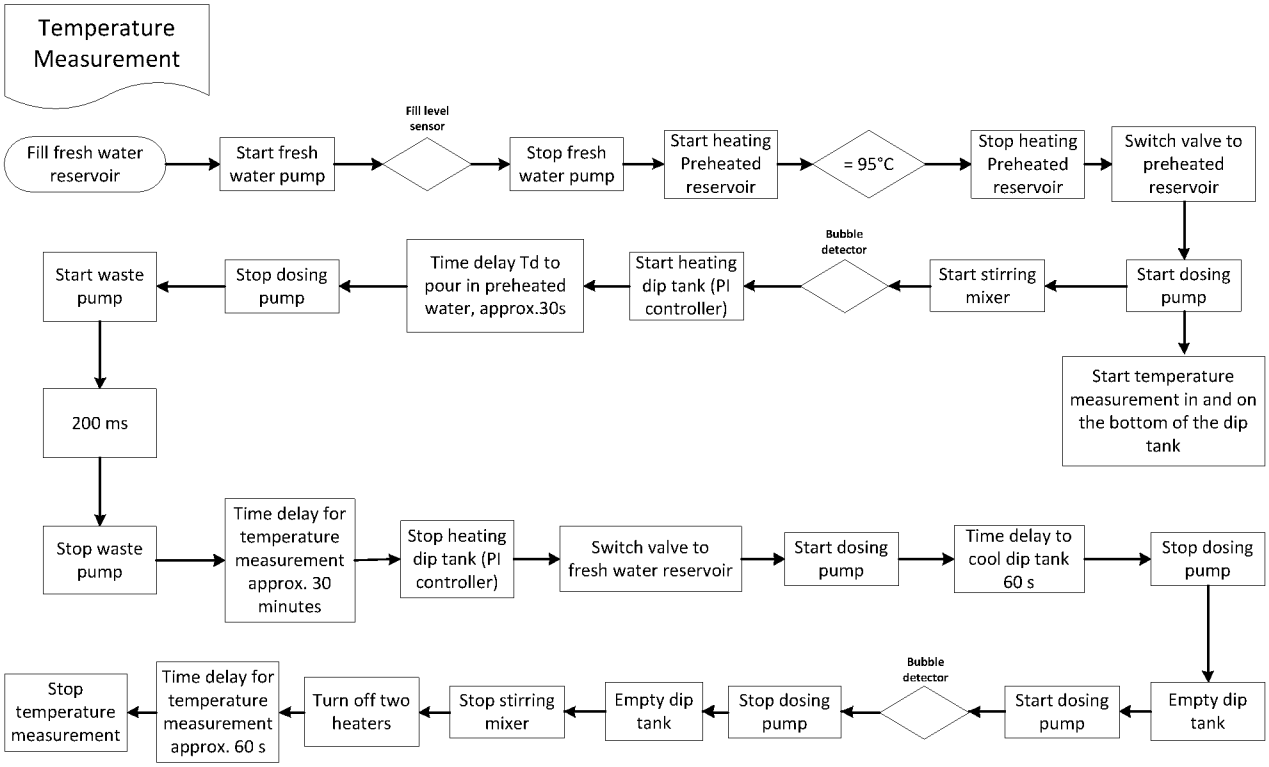
Temperature Measurement
Fill fresh water reservoir
Start fresh water pump
Fill level sensor
Stop fresh water pump
Start heating Preheated reservoir
= 95°C
Stop heating Preheated reservoir
Switch valve to preheated reservoir
Start dosing pump
Start temperature measurement in and on the bottom of the dip tank
Start stirring mixer
Bubble detector
Start heating dip tank (PI controller)
Time delay Td to pour in preheated water, approx.30s
Stop dosing pump
Start waste pump
200 ms
Stop waste pump
Time delay for temperature measurement approx. 30 minutes
Stop heating dip tank (PI controller)
Switch valve to fresh water reservoir
Start dosing pump
Time delay to cool dip tank 60 s
Stop dosing pump
Empty dip tank
Start dosing pump
Bubble detector
Stop dosing pump
Empty dip tank
Stop stirring mixer
Turn off two heaters
Time delay for temperature measurement approx. 60 s
Stop temperature measurement

Figure 4

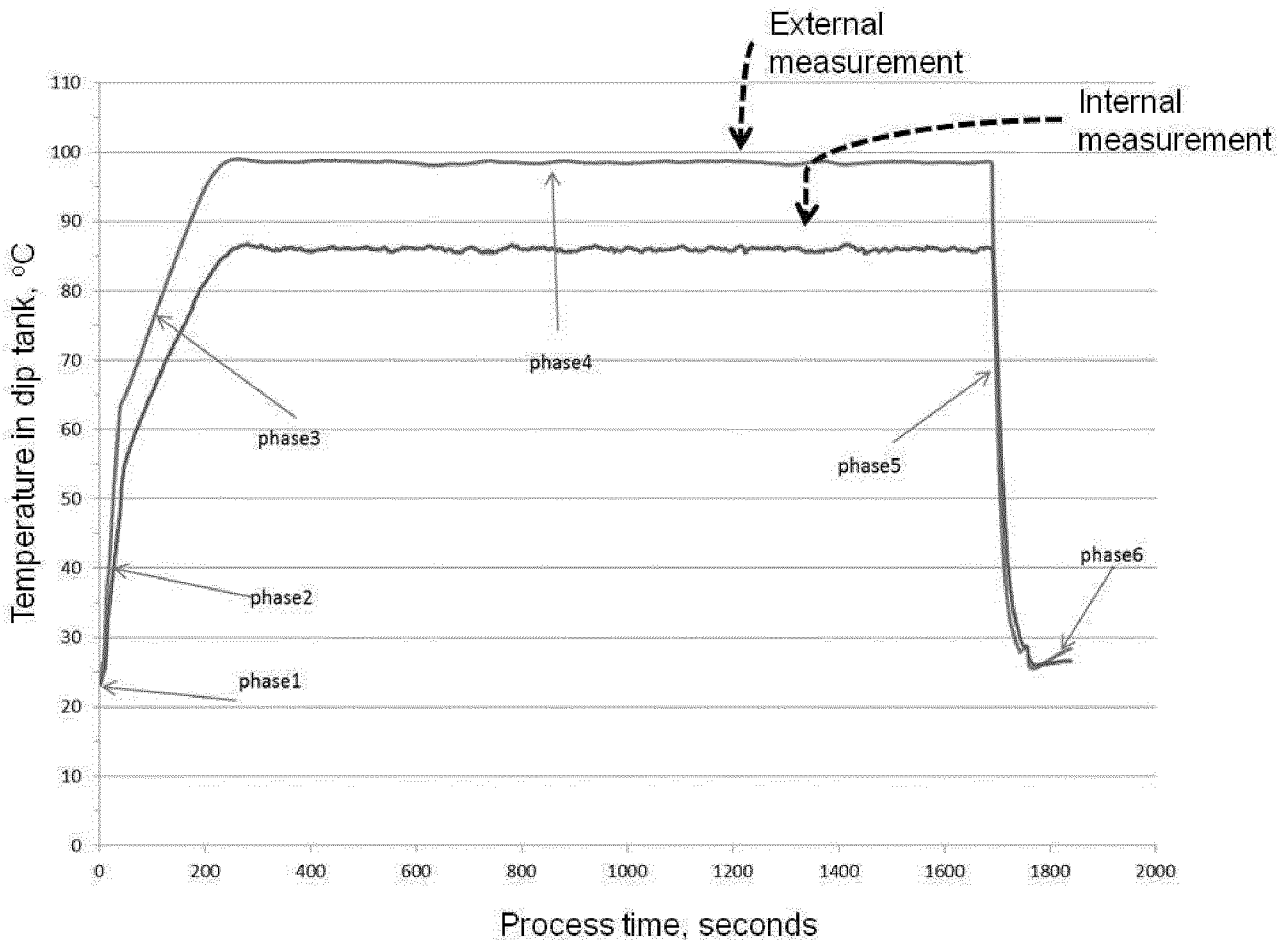
External measurement
Internal measurement
Temperature in dip tank, °C
Process time, seconds
phase1
phase2
phase3
phase4
phase5
phase6
0
10
20
30
40
50
60
70
80
90
100
110
0
200
400
600
800
1000
1200
1400
1600
1800
2000

Figure 5

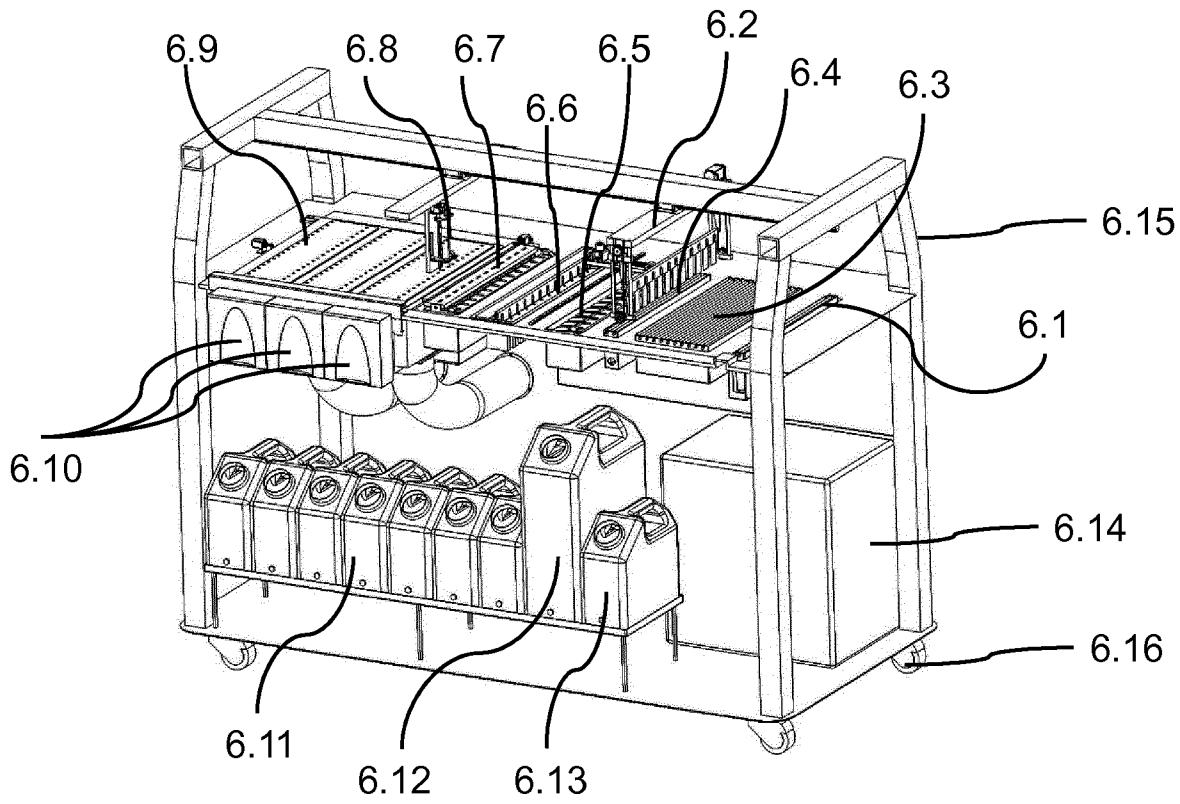

Figure 6

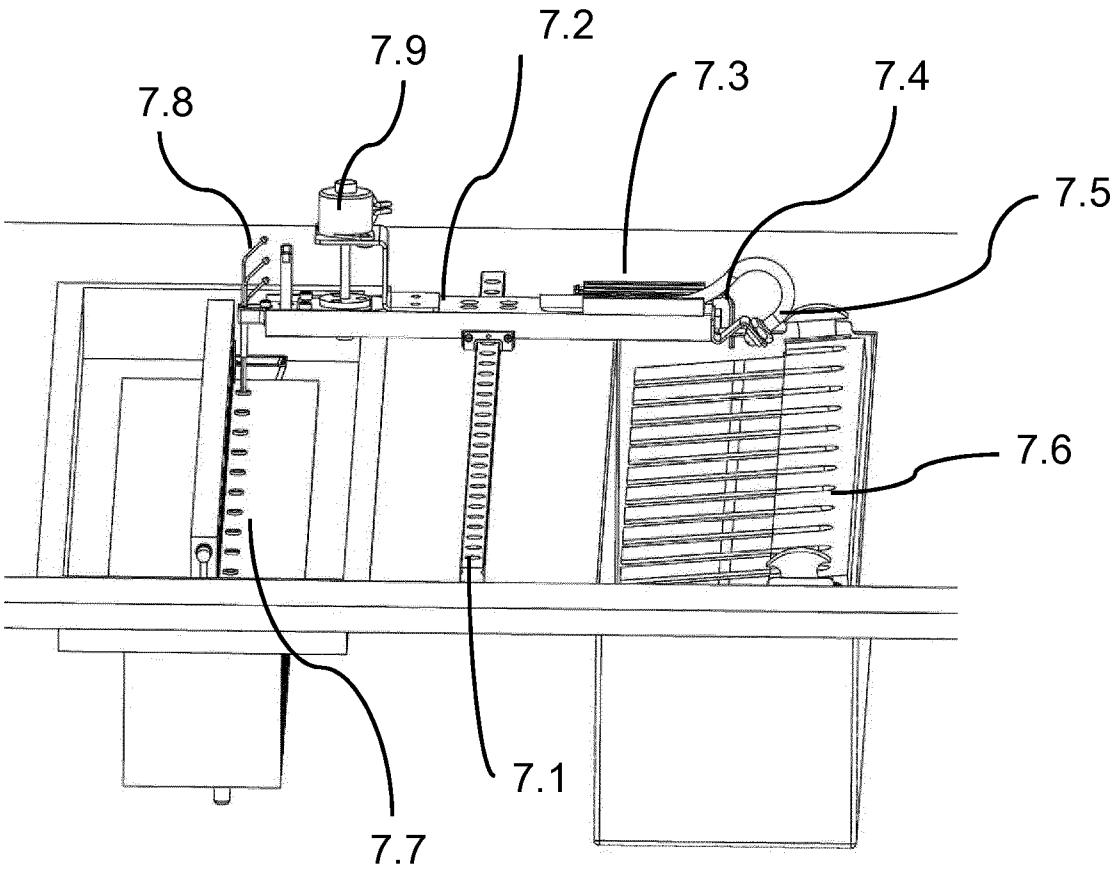

Figure 7

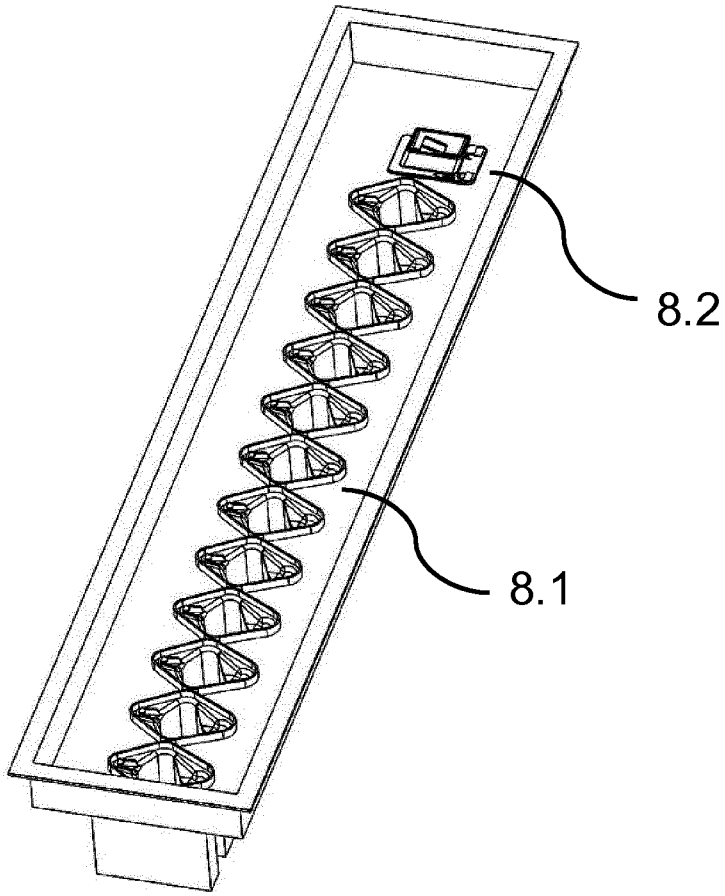

Figure 8

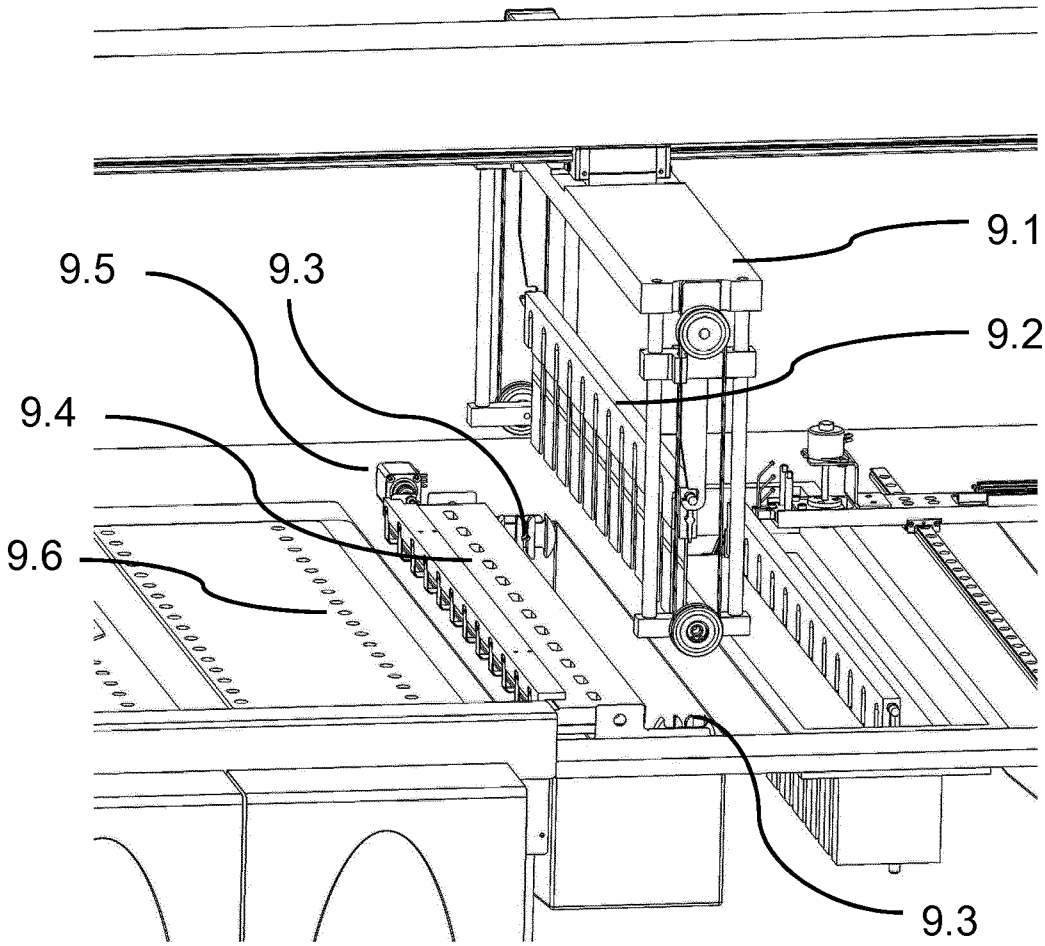

Figure 9

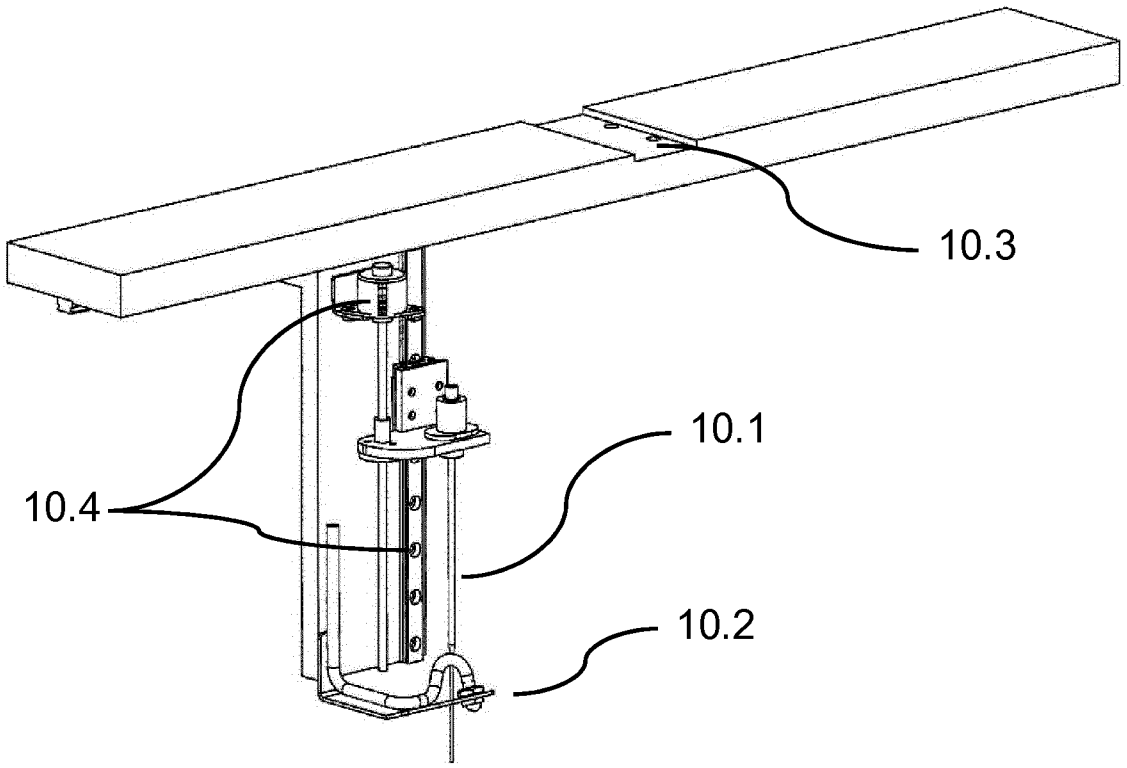

Figure 10

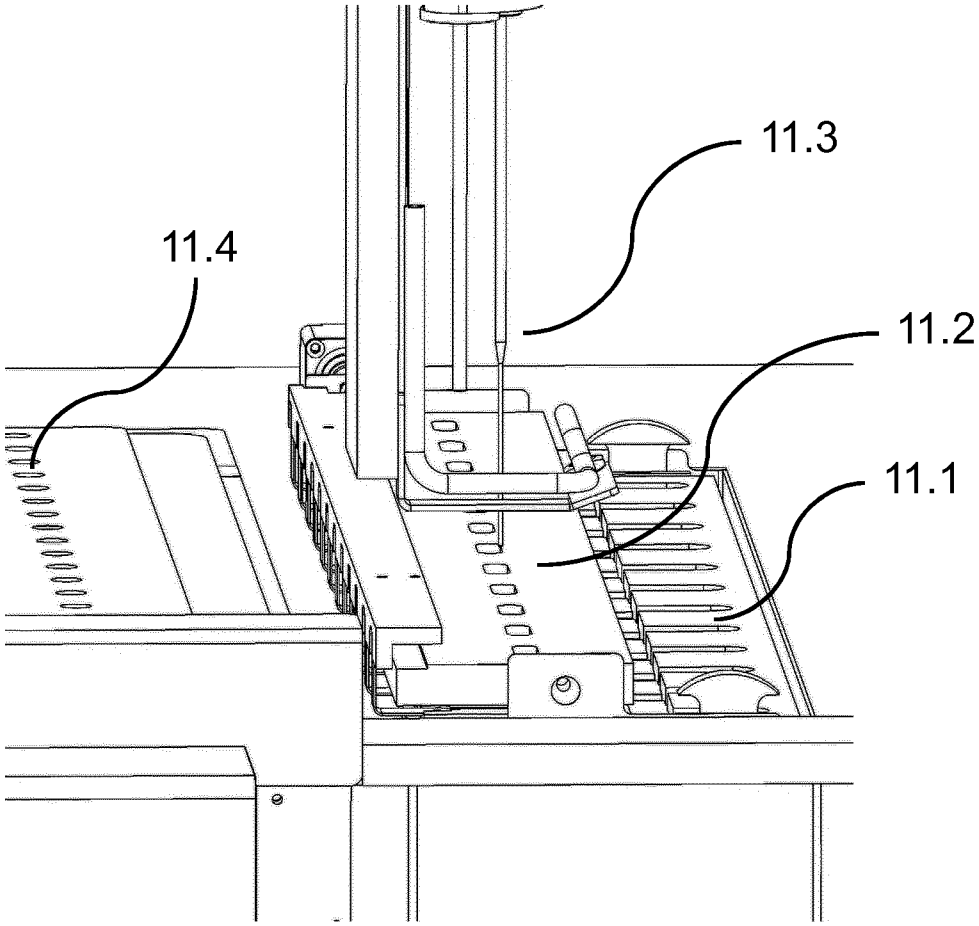

Figure 11

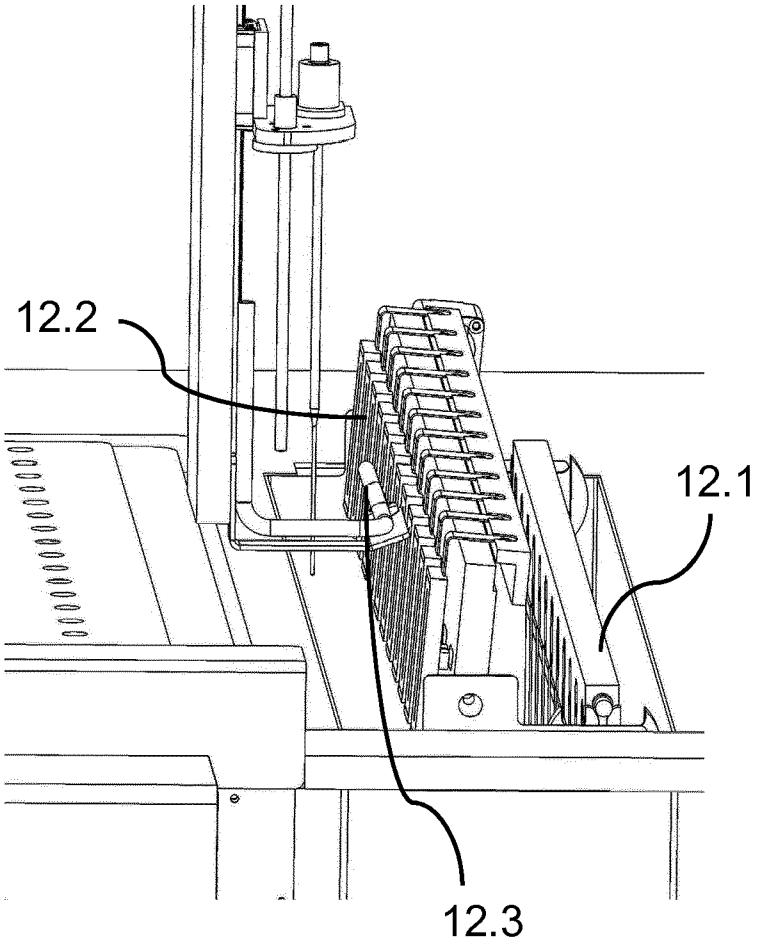

Figure 12

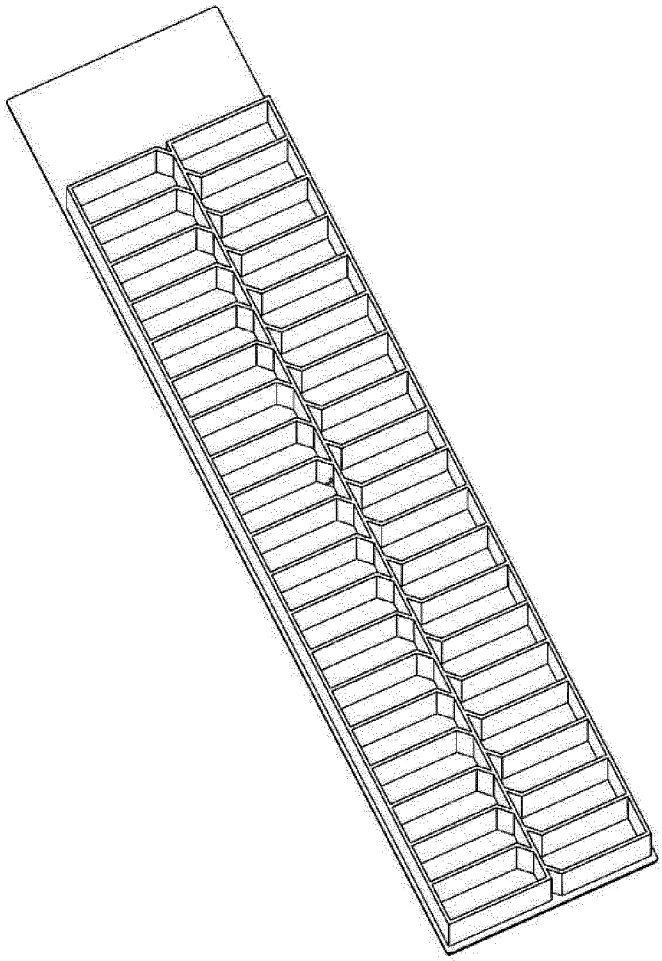

Figure 13

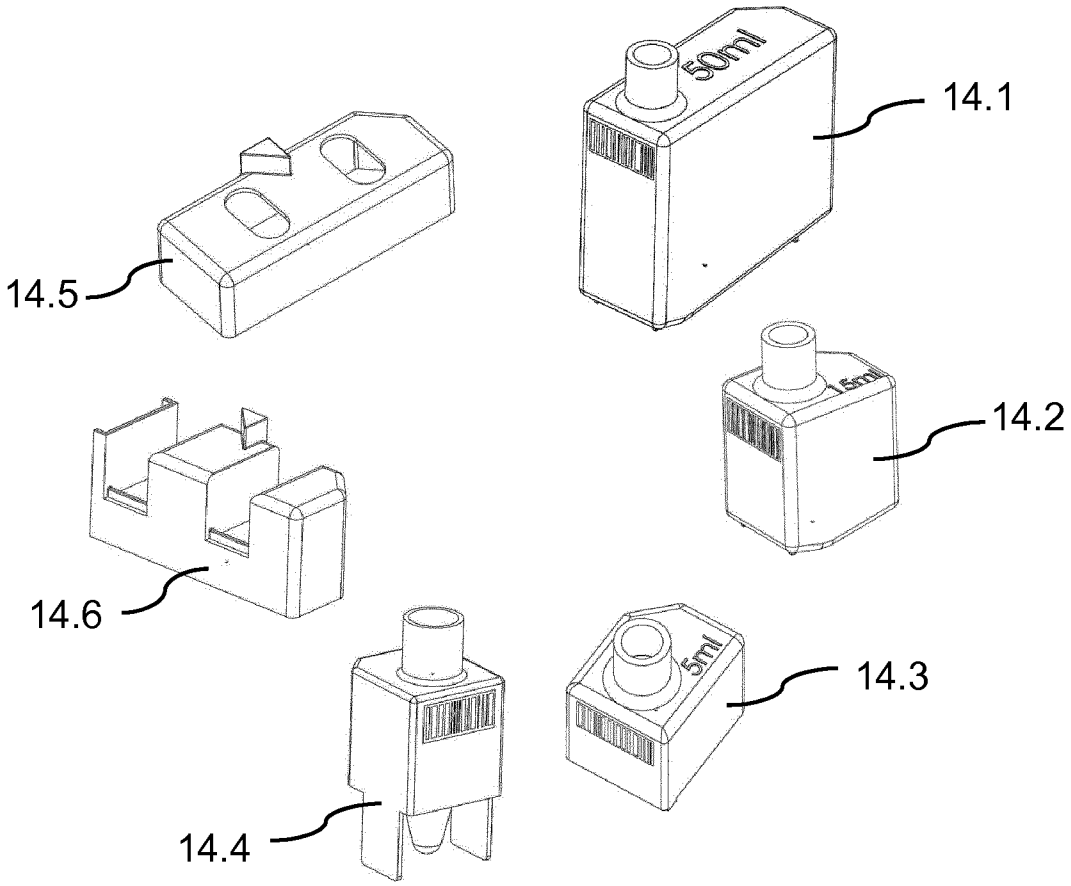

Figure 14

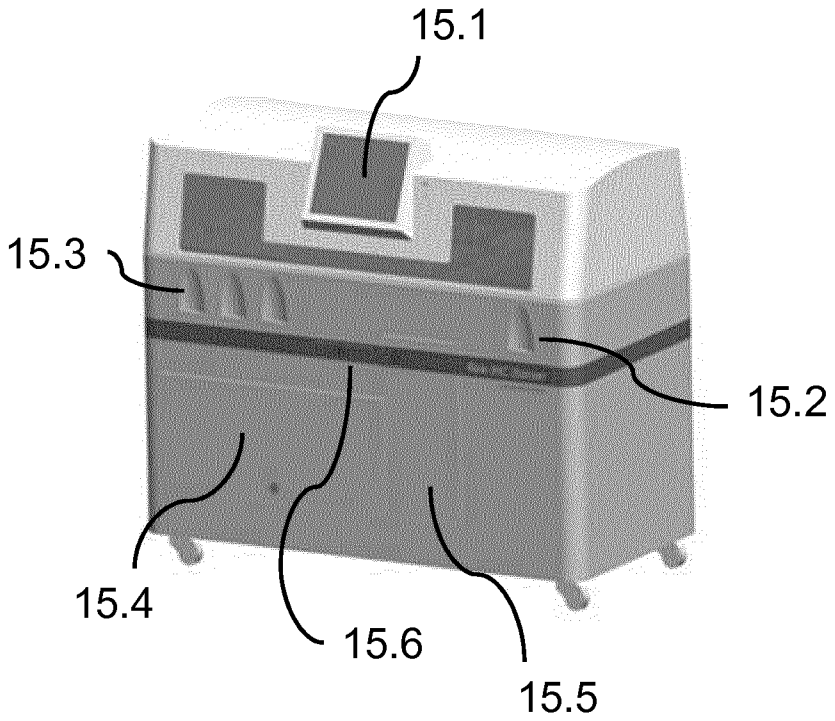

Figure 15

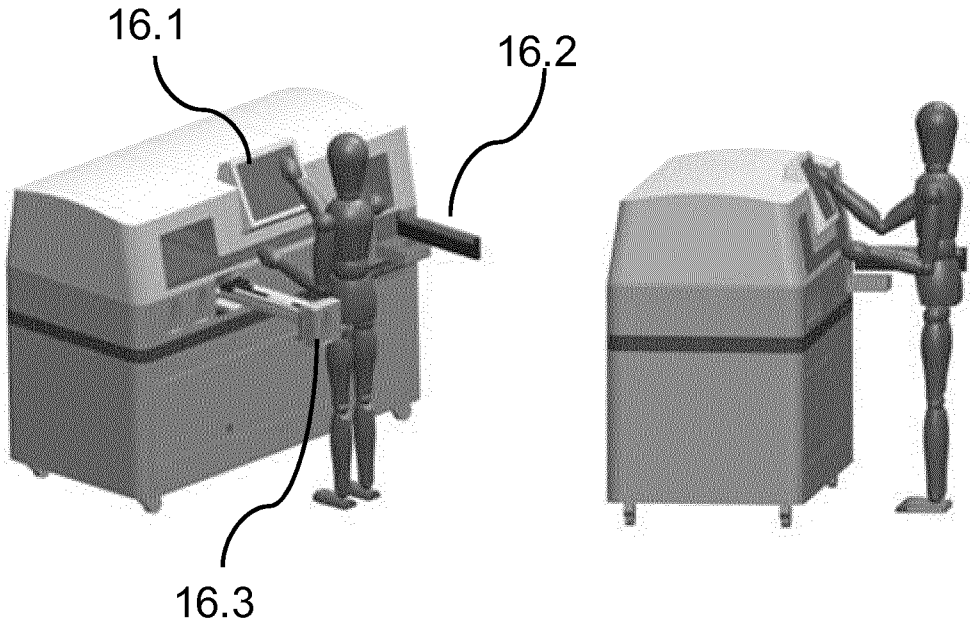

Figure 16

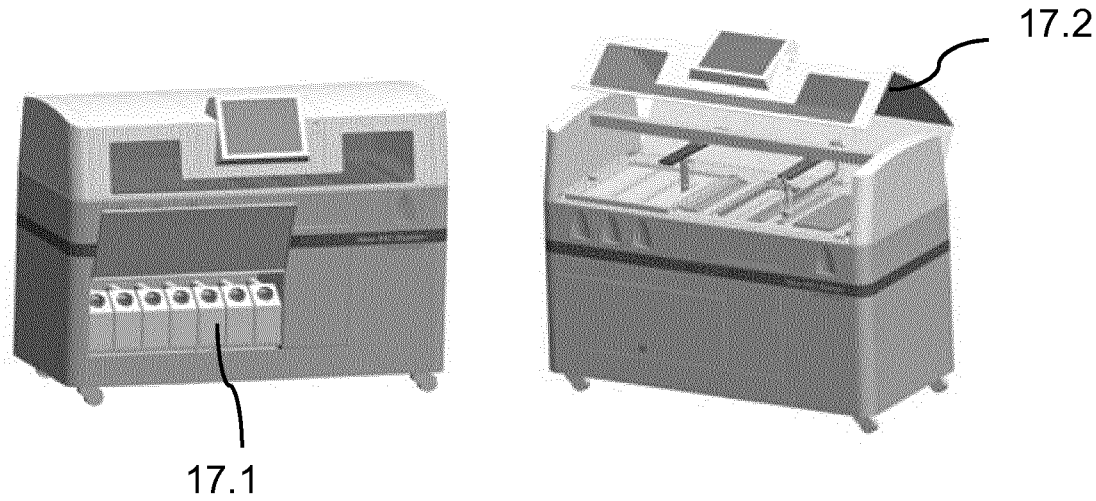

Figure 17