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Analytical Approach for the Optimization of Desiccant Weight in Rapid Test Kit Packaging: Accelerated Predictive Stability (APS)

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ABSTRACT

Present study involved determining the weight of cobalt-free desiccant (non-indicating) required in order to maintain the pregnancy test kit functionality during accelerated stability studies. The desiccant sachets were placed inside the aluminum pack to prevent damage to the immune chromatographic kits from humidity. The most of immune chromatographic test kits use indicating silica gel as a desiccant. The weight of silica gel in test kits ranges from 0.5 gm to 1.0 gm, which was used indiscriminately by manufacturers without any scientific rationality. The WHO has recommended verifying the desiccant condition before utilizing malaria test kits for diagnosis to identify the damage to the device. The non-indicating silica gel has the advantage of being cobalt-free, which is nontoxic. In comparison, non-indicating silica gel has the disadvantage of being unable to be identified by color change when packaging has leakage for ambient moisture, causing damage to the kit. The immune chromatographic test kits use antigen-antibody proteins for functioning, which are conjugated with a marker and coated on control and test line. High humidity can alter the functionality of proteins, conjugation, and affect the efficacy and efficiency (sensitivity and specificity) of the kits during the shelf life. Currently, the evidence of desiccants impact on kit stability is very minimal. Herein, we found that Weintraub and Tetreault's equation is the best method for calculating the optimum weight required for non-indicating silica gel. Utilizing this equation 1.5 gm of silica gel is the ideal weight required to sustain the sensitivity of the pregnancy test kit in 30^{0}C + 2^{0}C (temperature) and 75 % + 5 % (relative humidity) over 180 days in accelerated condition.

Keywords: Desiccant, Rapid test kit stability, Silica gel, Point-Of – Care, Lateral Flow packaging.

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Background

Immunoassay tests employed in industry, clinical or laboratory settings, doctor's offices and over-the-counter[1]. The pregnancy test kit is the most prevalent household over-the-counter tests that uses the immune-chromatographic technique[2]. In the pregnancy test kit alpha-hCG and beta-hCG, a monoclonal antibody was labeled with a gold nanoparticles marker. A typical pregnancy immune chromatography test kit consists of an inert backing material of polyvinyl chloride (PVC) packed within a plastic casing[1]. The other components of pregnancy immune chromatography test kits are glass fibers sample pad[3] and hydrophobic nitrocellulose acetate membrane (NCM) of certain pore size, onto which anti-target analyte are immobilized in a test & control line [4]. Also, the immune chromatography test kits contain glass fibers or non-woven fibers conjugate and absorbent pad [5, 6].

The immune chromatographic test kits are prepared by protein conjugation on gold nanoparticles (NPs), followed by NCM coating, drying, component assembly on inert PVC, slitting, and packing[1, 7].

The choice of packaging can have a very important influence on the immune chromatographic test kits stability[8]. For this reason, stability testing has to be performed in container closure systems representative of commercial or clinical products (ICH Q1A, 2003). Aluminum packets with specific thickness are used for kit packaging. To protect the kits from humidity the most often, desiccants are utilized. Desiccants commonly have many variants, including the silica gel, montmorillonite clay, calcium sulfate, molecular sieve, calcium oxide, and other adsorbents (activated alumina)[9].

Silica gel is a most commonly used for controlling the internal humidity of an immune chromatographic test kit and is approved by the United States FDA. Silica gel is natural mineral of silicon dioxide, which is converted by processing into a granular, vitreous, porous form and works on the principle of adsorption[9, 10]. The air water molecules are trapped by silica. The process of air water trapping is reversible, and the water molecules can be removed form silica after drying the silica at 80^{0}C[10]. Silica gel proficiently works at room temperature of about 20^{0}C to 32^{0}C and relative humidity (R.H.) of 60 % to 90% and can decreases the humidity in a container down to around 40% [9,10]. It can adsorb water up to 105^{0}C of temperature, but as temperature increases, its water adsorption capacity decreases[9].

Mainly silica gel desiccants are of two types, which are indicating (blue) and non- indicating (white) [11], as shown in Figure 1&2.

Figure 1. Blue-indicating Silica Gel

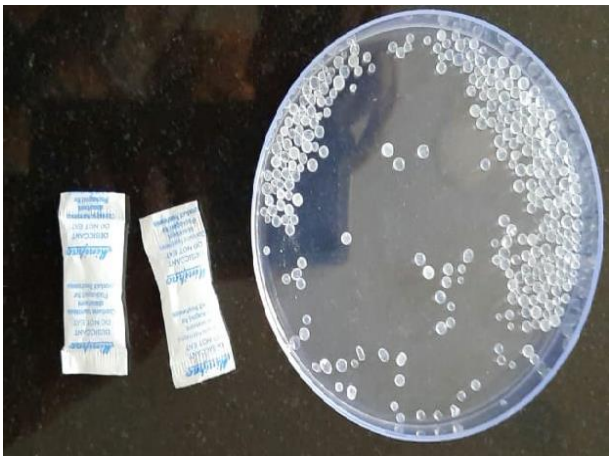


Figure 2. Non-indicating Silica Gel

Indicating silica gel is of two types, blue indicating and orange indicating[12]. The blue indicating silica gel beads are chemically treated with cobalt chloride at a concentration of 0.5 % to 1 % by weight to undergo color transition [9, 12, 13]. Cobalt chloride is blue in color, which turns from blue to purple and then to pink as it absorbs moisture from the air[9]. The white non-indicating silica gel is an insipid, moderate, nontoxic, non-corrosive, and chemically inert substance[14]. The immune chromatographic test kit degrades by extreme temperatures and humidity[15, 16, 17, 18]. In general, protection of kit from moisture damage is assured by kit packing in a sealed, impermeable aluminum pouch containing a silica gel that absorbs internal moisture[15, 18, 19]. The A examination of silica gel color variations has been recommended by the World Health Organization (WHO) to identify damage to kit by moisture following opening of the aluminum pouch of malaria test kit [15, 16].

Due to the prompt inspection nature, blue silica gel is commonly used. The EU, however, had used blue silica gel as a desiccant until 1998[12, 13]. Since 1998, blue indicating silica gel was superseded by orange color and by non-indicating silica gel [13]. However, manufacturers still are using indicating silica gel in pregnancy test kits packaging. The low cost and nontoxic nature of non-indicating silica gel rates it's higher than indicating silica gel [12,13,15,16].

In this work, we have attempted to assess the effect of white non-indicating silica gel on pregnancy test kit in accelerated stability and its ideal weight for maintaining stability. Herein, we have done accelerated stability study on pregnancy rapid test kits after packing the kits with different amount (grams) of non-indicating silica gel and studied the effects. Additionally, the well-known Weintraub and Tetreault equation's is used for calculating the optimum weight of silica gel desiccants.

Materials & Method

Materials: Stability Chamber of Osworld Scientific Equipments Pvt. Ltd. India and gifted panel of pregnancy immune chromatographic test kit manufactured by Seloi Healthcare India (brand name- Mom Quick Pregnancy Detection test) of lot number HCOM19007 is used in study. hCG 2000 I.U injection of Bharat Serum Vaccines ltd. India is used as standard specimen. 0.5 gm sachet of non-indicating silica gel (white) manufactured by Cilicant Chem Pvt.Ltd. India of batch number SGB0.5G/1910-04 is used as desiccant.

Method

Accelerated Stability Study Design:

The stability of immune chromatographic test kits (*In-vitro* diagnostic) reflects the performance characteristics over a defined time interval in particular storage conditions [14, 21, 22].

The stability of the product is affected by various internal and external product components in which silica gel (desiccants) is also vital. Manufacturers of immune chromatography test kits uses stability tests to ascertained shelf life and in-use life of products [22, 23].

Accelerated stability studies were designed to increase the rate of chemical and physical degradation or change by using environmental stress conditions. The design of an accelerated stability evaluation includes stress conditions of temperature and humidity [21,22,24,25].

Herein, the following parameters were considered for conducting the accelerated stability study on pregnancy test kits[22, 26].

a) The typical storage conditions recommended by manufacturers for products are 2^0C - 30 ^{0}C. The accelerated predictive study were performed under 30^{0}C + 2^{0}C and 75% + 5% of temperature and relative humidity condition respectively, which are taken into account after analyzing the manufacturer's recommended normal storage conditions and according to ICH Guidelines[27,28]. An appropriate product handling procedure was understood before opening the pouch of the kit. The pregnancy kit was opened in the dehumidifier room, with relative humidity (RH) below 20 % and temperature below 20 ^{0}C.
b) The pregnancy test kit was kept for six months under stability. The kits were tested in stability in three-time interval. The kit for initial baseline is evaluated at 1st month, followed with 3rd month and 6th month evaluation[22].
c) The results specifications were designed for study to identify out of specification results while kit evaluation. The three pregnancy test kits were tested for sensitivity testing utilizing 100 mIU/ml hCG specimen sample, and two test kits were tested for negative specimen. If two pink-purple colored lines appear in the control region (C) and test region (T), the results are considered positive. The grading was given for line color intensity using RGB and HSV color models. The +1 relegation was a low-intensity test color band, +2 was a medium - intensity color band, and +3 was a high-intensity color band. The results were considered invalid if the control line fails to appear. The test kits should be positive when tested by positive

samples and show a negative result when tested by negative samples[1].

d) Before kit evaluation, the sealed test pouch and specimen samples were allowed to reach room temperature. Now the kits were removed from the sealed pouch and then 40 ml drops of positive and negative specimen's samples were integrated into the sample pad. The kit was laid on a flat surface with the result window facing up. The colored band was optically interpreted after 3 minutes. However, to corroborate the negative result reaction time of 10 minutes is taken into count.

e) The references standards specimen's samples were prepared by diluting market available hCG injection to negative urine.

The Optimum Weight of Non-Indicating Silica Gel

RD silica gel of Art-Sorb has recommended 20 kg/m^3 to 0.5 kg/m^3 of silica gel weight as optimum[29, 31]. Thomson determined 20 kg/m^3 of customary density silica gel enough to buffer the humidity in a full year without reconditioning[29, 30].Also various online sites calculate the amount of silica gel required. The measurement was predicated upon uploading the data related to packing volume, differential RH, air exchange per day, and days between humidity maintenance etc. Small Corp (Figure. 3), SCP silica gel products (Figure 4), ProEx Protection expert Australia (Figure 5) are the examples of few online websites[32, 33, 34]. These sites can be utilized to calculate the amount of silica gel required for humidity protection, but on what basis this calculation works is certainly unknown. Therefore, we utilized the well-accepted equation of Weintraub and Tetreault to calculate the quantity of desiccants required for pregnancy immune chromatographic test kit.

Figure 3. Small Corp site showing silica gel weight calculation

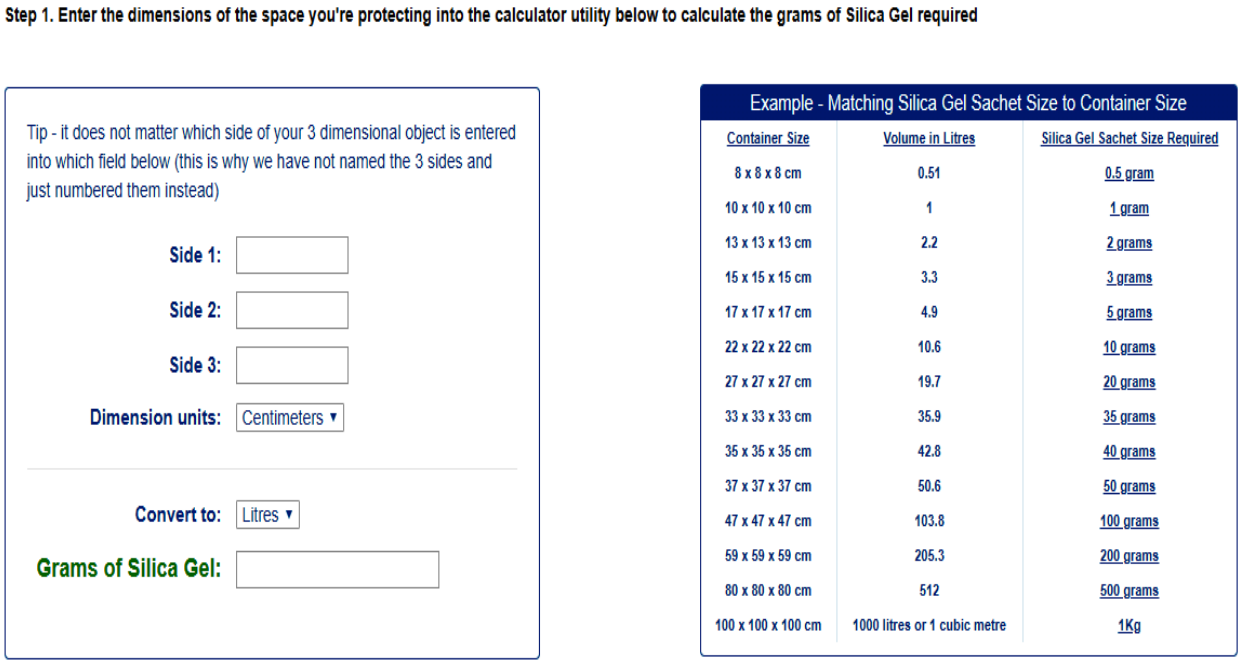


Figure 4. SCP silica gel product site showing silica gel weight calculation

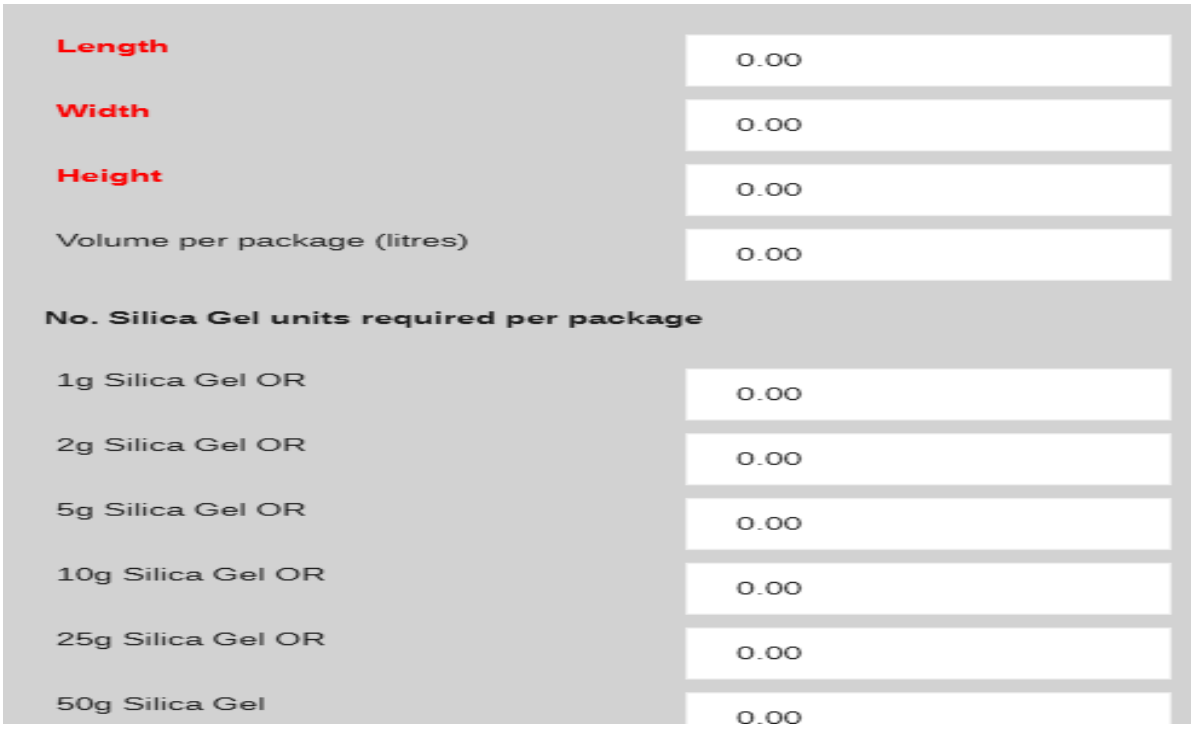


Figure 5. ProEx Protection expert Australia site showing silica gel weight calculation.

To find exactly the amount of silica required, we first have to identify the buffering capacity of silica gel[29]. In this study, we used a white non-indicating silica gel with an apparent density approx. 750 g/l. The silica gel has beads of the size 3 - 6 mm and is globular in shape, which allows the air to pass more quickly. The internal surface areas of these beads were approximately 800 m^2/g. The absorption capacity of this silica gel was determined in percentage by weight at 30°C ± 2°C temperature and relative humidity (RH) of 75 % ± 5 %.

The Buffering Capacity of Silica gel

Buffering capacity is the capacity of 1 kilogram of silica gel to gain or lost the water amount (in grams) for 1% change in relative humidity (RH) [29, 30]. For example, if 50 grams of moisture is adsorbs by 1 kilogram of silica gel between 40-50% RH than, M is calculated as

M = 50 grams of moisture divide by 10%, RH = 5

The EMC/RH points on isotherm, RH magnitude to determine M and measurement along the adsorption/desorption isotherm are factors, which causes variation in M[29].

Modified value Mh (variable) was used to avoid confusion, which is corrected form of M. The Mh is the average water

amount (grams) that is gained / lost in 1 % change in relative humidity (RH) by 1 kilogram of silica gel after calculated by repeated cycling of adsorption and desorption within a specific relative humidity (RH) range until a constant value is obtained. After determining experimentally, the average absorption capacity of white non-indicative silica gel at 25°C ± 2°C , RH of 75 % ± 5 % is 37 %, was found Mh is 37, which corresponds to the supplier (COA = 37.25 %) certificate of analysis (Figure 6). The Mh and absorption capacity were calculated on average by repeated charging the silica gel beads at 80°C for four hours.

Test	Specifications	Results
Visual Inspection	The pouches should be clean (i.e free from dust, any spot or foreign particles)& sealing should be leak proof	Complies
Loss On Drying (@150 ± 5 ° C For 4 Hrs)	Not more than 5 %	1.67 %
Moisture Adsorption Capacity (@Temp.25±2° C and RH 75±5 %)	Not less than 27 %	37.25 %
Pouch Durability- Strength of pouch	The pouch should not show bursting, puncturing or other failure	Complies
Printed Matter	Printed matter should be readable and as per mentioned below(Additions allowed) CILICANT DESICCANT DO NOT EAT	CILICANT SILICA GEL DESICCANT DO NOT EAT
Weight Net Weight Gross Weight	 0.5 ± 0.2 gm 0.6 ± 0.2 gm	 0.42 gm 0.51 gm

Figure 6. Supplier COA of Silica gel desiccant

Determining the Required Silica Gel Quantity by Weintraub and Tetreault Equation:

To estimate the amount of silica gel required to buffer RH within an aluminum pouch must have to take the varying conditions of the environment and buffering capacity of material into account. The amount of silica gel required after considering the varying condition the Weintraub and Tetreault equation is used as[35].

$$Q = \frac{(Ceq\ D)\ X\ V\ X\ (N\ t)}{(Mh\ X\ F)}$$

Where: Q = Silica gel weight required in grams.

Ceq = Water vapors concentration at saturation e.g. at 30^0 C, a cubic meter of air holds 30 grams of water vapor at saturation.)

D = Differential between the external humidity and humidity within the aluminum pouch. Here, the pregnancy test kit was repacked at 30 % RH, and the external environmental condition in the stability chamber was 75 % (median). Therefore, the difference D = 45 % or 0.45

V = Aluminum pouch volume, expressed in cubic meters. In this case, we used aluminum pouches of length 120 mm, width 65 mm, and thickness 0.030 mm. The total volume was 234 mm^3 or 0.234 m^3.

N = Air exchanges/day number. One air exchange / day was used by Thomson used a for a typical moderately sealed exhibit case[30].

t = Maximum number of days that the pouch is kept. In this case, we used 180 days (6 months).

Mh = Buffering capacity of silica gel within the R.H. range in use, taking hysteresis into account. In this case, Mh was 37.

F = Maximum acceptable range of RH fluctuation. In this, we are taking account maximum RH variation of the stability chamber (70 %-80%). Therefore, F is 10 in this case.

Now, using the formula the Q is

$$Q = \frac{(30\ X\ 0.45) X\ 0.234\ X\ (1X\ 180)}{(37\ X\ 10)} = \mathbf{1.5\ gm}$$

According to the Weintraub and Tetreault equation, 1.5 gm of non-indicating silica gel desiccant was the optimum quantity required for humidity buffering. The most of pregnancy test kit's market available sample uses 0.5 gm and 1.0 gm of silica gel sachet as desiccant. The regular utilized 0.5 gm and 1.0 gm (marketed amount) of silica gel was compared with 1.5 gm (calculated amount) of silica gel to ascertain their effects on pregnancy test kit performance. In this study, we utilized 1.5 gm of silica gel sachet (3 sachets) to compensate for damage by leakage of packing.

Reference Standard Specimen Panel Selection

The reference standard includes well-characterized specimens utilized for interpreting the results [22]. The pregnancy test kit gives positive results if human chorionic

gonadotropin (hCG) is present in female urine. Human chorionic gonadotropin (hCG) is a hormone secreted by the placenta after conception (when the sperm fertilizes the egg) [27].

It takes about a fortnight for hCG levels to be high enough to be detected in the urine. The pregnant women after about four weeks of LMP (average one week before the first missed period) had 25 mIU /ml - 750 mIU/ml concentration of hCG in urine. Non-pregnant women have less than 10 mIU / ml [27]. In borderline pregnancy hCG concentration was 10 mIU/ml - 25 mIU /ml. A pregnant woman has a hCG more than 25 mIU /ml concentration[27].

In this study, we used a specimen sample of 100 mIU/ml hCG concentration for testing. The 100 mIU/ml was regarded as the average range of hCG concentration present at about four weeks after the last menstrual period or LMP. The specimen samples were prepared by diluting the hCG injection (Figure 7) of level 2000 IU to 100 mIU/ml in negative urine.

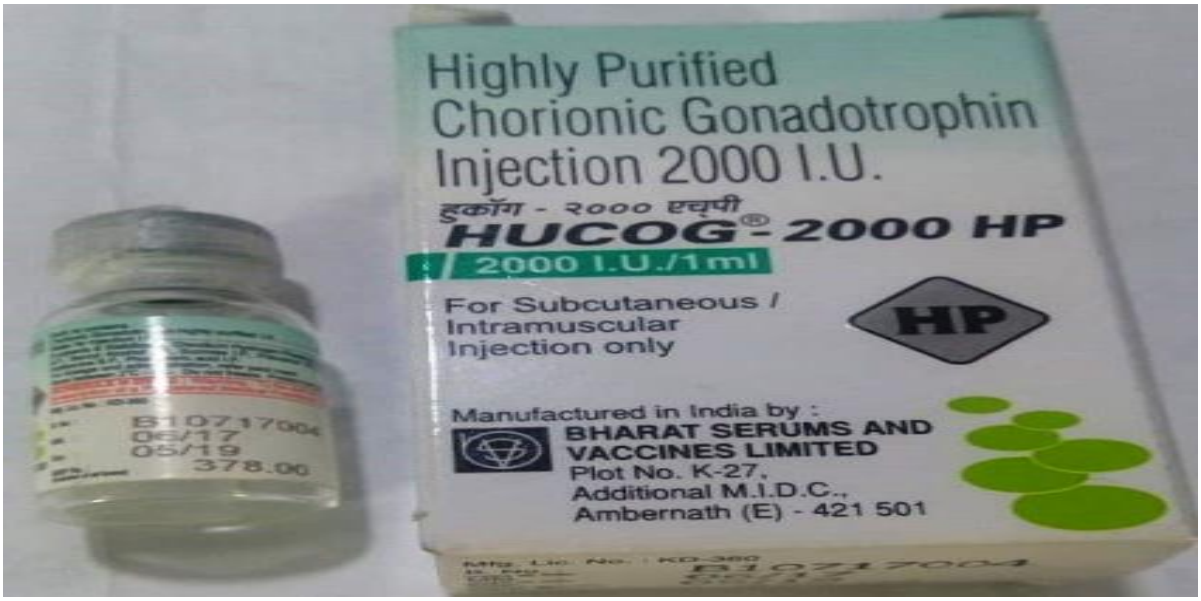


Figure 7: hCG injection of concentration 2000 IU.

The specimen samples were stable for one week. The specimen samples were freshly prepared every time, for testing.

Pregnancy Immune Chromatographic Test Kit Panel Selection

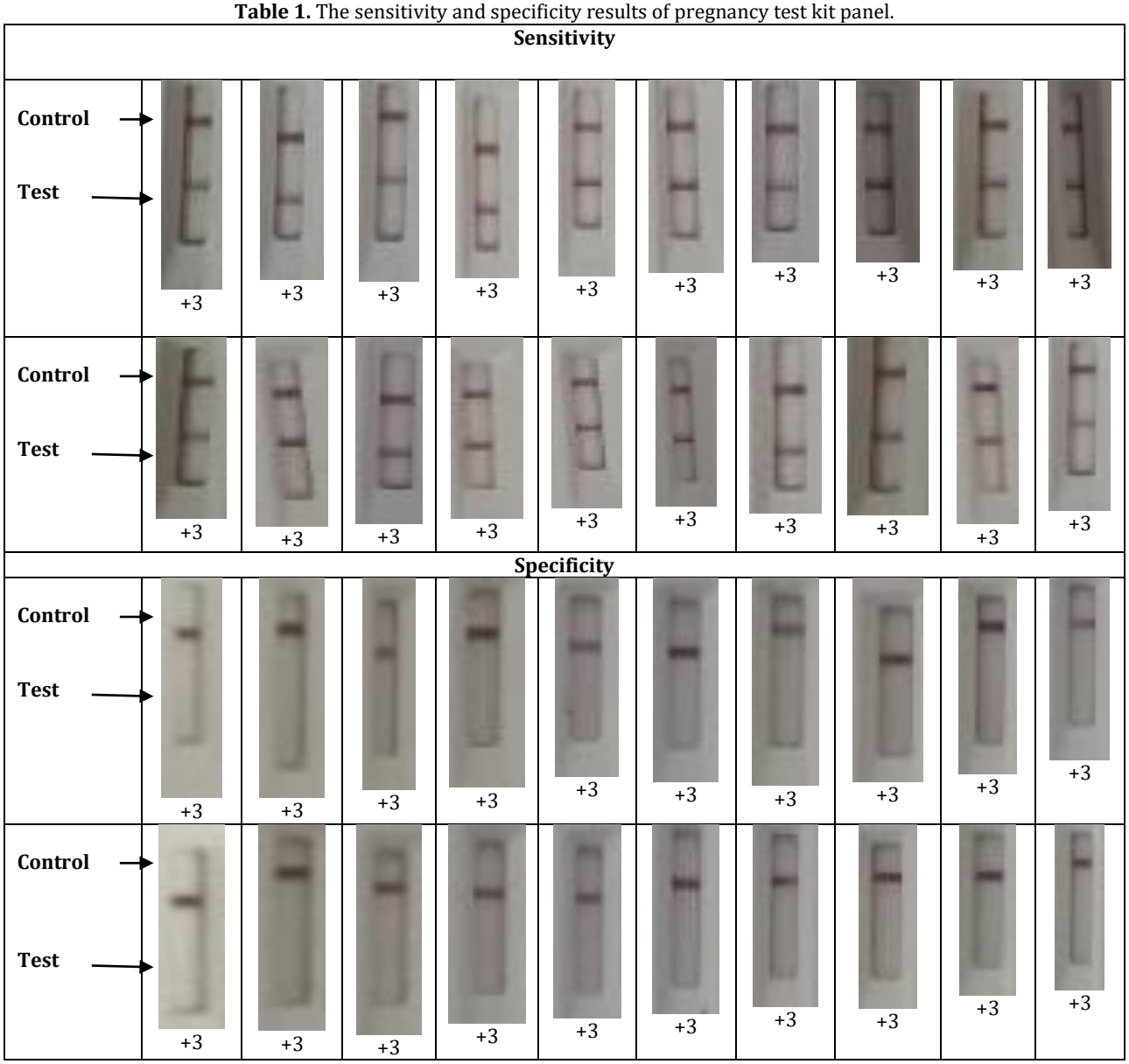
The different batches of Seloi Healthcare, pregnancy test kits (Figure 8) were evaluated for uniformity. The kits were selected in such a way to avoid the variation caused by other components (protein conjugation and coating). Pregnancy test kits of lot no. HCOM19007 was used for the study.

Figure 8: Pregnancy test kit sample panel of lot no. HCOM19007.

This selected pregnancy test kits batch results found uniform with a +3 (high) band intensity. Additionally, the selected test kits were tested by negative urine specimen samples to analyze its specificity and the results were

found uniform without any false positive effect. The selected pregnancy test kits panel sensitivity and specificity uniformity results were shown in Table 1.

Table 1. The sensitivity and specificity results of pregnancy test kit panel.



Sample Re-Packing

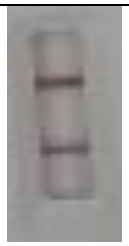
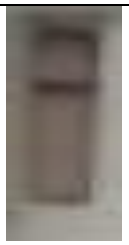
The selected panels of pregnancy test kits were repacked in the room, having 30 % (RH) and 25^{0}C (temperature). Twenty-one test kits each were repacked with 0.5 gm, 1.0 gm, and 1.5 gm of non-indicating silica gel sachet. Out of 21, sensitivity testing was performed on 15 test kits, and a specificity test were performed on 6 test kits.

The 21 test kits for each weight were kept under accelerated stability conditions (25°C + 2^{0}C and RH of 75 % + 5 %) for six months. The test kits were evaluated at the 1st, 3rd, and 6th month of time intervals.

Results

The accelerated stability samples of the pregnancy test kits were evaluated and the results are tabulated in Table 2, 3, and 4. The accelerated stability results indicate, 0.5 gm (Table 2) white non-indicating silica with a buffering capacity (Mh) of 37, have maintained the band intensity in the 1st month's analysis (sensitivity and specificity). On 3rd month, the one test kit (out of five) band intensity decreased to +2 (medium) in the sensitivity analysis. Also, on the same month, two test kits (out of five) had lower band intensity of +2 in specificity analysis. On 6th month, accelerated stability testing out of the five test kits, four test kit's control and test line band intensity decreased from +3 (high) to +1 (low) in a sensitivity analysis. In the specificity analysis, out of five test kits, the control band intensity of three test kits decreased to +1, one test kit has a +2-control band intensity and one test kit has maintained its control band intensity of +3. The control line band intensity in one test kit out of five decreased to +2 at six months. No test kit has shown false results. The silica weight does not have any effect in engendering false results, but at the same time, it shows effects on the kit's sensitivity (band intensity).

Table 2. Accelerated Stability Results of Pregnancy Test Kit packed with 0.5 gm of silica.

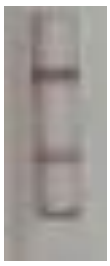
Parameters	1st Month Accelerated Stability				
	Kit No. 1	Kit No. 2	Kit No. 3	Kit No. 4	Kit No. 5
Sensitivity Control → Test →	 +3	 +3	 +3	 +3	 +3
Specificity Control → Test →	 +3	 +3	 +3	 +3	 +3
Parameters	**3rd Month Accelerated Stability**				
	Kit No. 1	**Kit No. 2**	**Kit No. 3**	**Kit No. 4**	**Kit No. 5**
Sensitivity Control → Test →	 +3	 +3	 +3	 +3	 +2
Specificity Control → Test →	 +2	 +3	 +3	 +3	 +2
Parameters	**6th Month Accelerated Stability**				
	Kit No. 1	**Kit No. 2**	**Kit No. 3**	**Kit No. 4**	**Kit No. 5**
Sensitivity Control → Test →	 +1	 +1	 +1	 +1	 +3

Specificity **Control →** **Test →**	 +1	 +1	 +1	 +3	 +2

The test and control line band intensity for 1.0 gm (Table 3) white non-indicating silica did not decrease at the 1st and 3rd month's stability testing. Only one test kit found with a decremented control band intensity of +2 at the 6th month specificity testing. It was noticed that non-indicating silica does not give the false results.

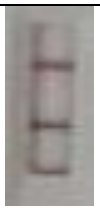
Table 3. Accelerated Stability Results of Pregnancy Test Kit packed with 1.0 gm of silica.

Parameters	**1st Month Accelerated Stability**				
	Kit No. 1	**Kit No. 2**	**Kit No. 3**	**Kit No. 4**	**Kit No. 5**
Sensitivity **Control →** **Test →**	 +3	 +3	 +3	 +3	 +3
Specificity **Control →** **Test →**	 +3	 +3	 +3	 +3	 +3
Parameters	**3rd Month Accelerated Stability**				
	Kit No. 1	**Kit No. 2**	**Kit No. 3**	**Kit No. 4**	**Kit No. 5**
Sensitivity **Control →** **Test →**	 +3	 +3	 +3	 +3	 +3
Specificity **Control →** **Test →**	 +3	 +3	 +3	 +3	 +3

Parameters	6th Month Accelerated Stability				
	Kit No. 1	Kit No. 2	Kit No. 3	Kit No. 4	Kit No. 5
Sensitivity Control → Test →	 +3	 +3	 +3	 +3	 +3
Specificity Control → Test →	 +3	 +3	 +3	 +3	 +2

However, the 1.5 gm (Table 4) of the white non-indicating silica gel maintained the band intensity of the test and control lines throughout the stability. At 1.5 gm weight, no false results were noticed. It shows that the non-indicating silica gel of 1.5 gm is the optimum weight required for maintaining the high band intensity (+3) of pregnancy test kits in accelerated stability study for 180 days at 30 ^{0}C + 2^{0}C (temperature) and 75 % + 5 % (relative humidity) conditions. The weight calculated by the Weintraub and Tetreault equation gives the exact amount of non-indicating silica gel weight required to compensate for the performance of the kit loss by environmental humidity.

Table 4. Accelerated Stability Results of Pregnancy Test Kit packed with 1.5 gm of silica.

Parameters	1st Month Accelerated Stability				
	Kit No. 1	Kit No. 2	Kit No. 3	Kit No. 4	Kit No. 5
Sensitivity Control → Test →	 +3	 +3	 +3	 +3	 +3
Specificity Control → Test →	 +3	 +3	 +3	 +3	 +3
Parameters	**3rd Month Accelerated Stability**				
	Kit No. 1	**Kit No. 2**	**Kit No. 3**	**Kit No. 4**	**Kit No. 5**
Sensitivity Control → Test →	 +3	 +3	 +3	 +3	 +3

Specificity **Control →** **Test →**	 +3	 +3	 +3	 +3	 +3
Parameters	**6th Month Accelerated Stability**				
	Kit No. 1	**Kit No. 2**	**Kit No. 3**	**Kit No. 4**	**Kit No. 5**
Sensitivity **Control →** **Test →**	 +3	 +3	 +3	 +3	 +3
Specificity **Control →** **Test →**	 +3	 +3	 +3	 +3	 +3

Conclusion

In this case study, it was found that underweight silica gel can affect the kits test and control band intensity. Using underweight non-indicating silica gel as desiccants can decrease the sensitivity from high to low. However, the non-indicating silica gel weight does not have any effect on specificity (false result). The Weintraub and Tetreault equation is useful in the calculation of weight required for desiccants in immune chromatographic test kits. The silica gel buffering capacity, volume of the packing pouch, external environmental conditions, packing leakage, and shelf life are vital variables for deciding the weight of silica gel required. The Weintraub and Tetreault equation address all these variables for calculating the weight of the desiccant. At the user end, the non-indicating silica gel conditions are challenging to identify visually. Therefore, the manufacturers have to use the optimum silica gel weight for maintaining the performance of the immune chromatographic test kit.

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